

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	446	(544/126).CCLS.	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2005/11/01 12:06
S2	6	((("6515005") or ("6387937") or ("6888004"))).PN.	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2005/11/01 07:58
S3	552	(546/82).CCLS.	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2005/11/01 09:11

Connecting via Winsock to STN

Welcome to STN International! Enter x:x

LOGINID:SSSPTA1600RXA

PASSWORD:

TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * Welcome to STN International * * * * *

NEWS 1 Web Page URLs for STN Seminar Schedule - N. America
NEWS 2 "Ask CAS" for self-help around the clock
NEWS 3 JUL 20 Powerful new interactive analysis and visualization software,
STN AnaVist, now available
NEWS 4 AUG 11 STN AnaVist workshops to be held in North America
NEWS 5 AUG 30 CA/CAPLUS - Increased access to 19th century research documents
NEWS 6 AUG 30 CASREACT - Enhanced with displayable reaction conditions
NEWS 7 SEP 09 ACD predicted properties enhanced in REGISTRY/ZREGISTRY
NEWS 8 OCT 03 MATHDI removed from STN
NEWS 9 OCT 04 CA/CAPLUS-Canadian Intellectual Property Office (CIPO) added
to core patent offices
NEWS 10 OCT 06 STN AnaVist workshops to be held in North America
NEWS 11 OCT 13 New CAS Information Use Policies Effective October 17, 2005
NEWS 12 OCT 17 STN(R) AnaVist(TM), Version 1.01, allows the export/download
of CAPLUS documents for use in third-party analysis and
visualization tools
NEWS 13 OCT 27 Free KWIC format extended in full-text databases
NEWS 14 OCT 27 DIOGENES content streamlined
NEWS 15 OCT 27 EPFULL enhanced with additional content

NEWS EXPRESS JUNE 13 CURRENT WINDOWS VERSION IS V8.0, CURRENT
MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
AND CURRENT DISCOVER FILE IS DATED 13 JUNE 2005

NEWS HOURS STN Operating Hours Plus Help Desk Availability
NEWS INTER General Internet Information
NEWS LOGIN Welcome Banner and News Items
NEWS PHONE Direct Dial and Telecommunication Network Access to STN
NEWS WWW CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that specific topic.

All use of STN is subject to the provisions of the STN Customer agreement. Please note that this agreement limits use to scientific research. Use for software development or design or implementation of commercial gateways or other similar uses is prohibited and may result in loss of user privileges and other penalties.

* * * * * STN Columbus * * * * *

FILE 'HOME' ENTERED AT 06:33:54 ON 01 NOV 2005

=> fil reg

COST IN U.S. DOLLARS

SINCE FILE
ENTRY

TOTAL
SESSION

FULL ESTIMATED COST

0.21

0.21

FILE 'REGISTRY' ENTERED AT 06:34:02 ON 01 NOV 2005

Best Available Copy

USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2005 American Chemical Society (ACS)

Property values tagged with IC are from the ZIC/VINITI data file
provided by InfoChem.

STRUCTURE FILE UPDATES: 30 OCT 2005 HIGHEST RN 866393-44-4
DICTIONARY FILE UPDATES: 30 OCT 2005 HIGHEST RN 866393-44-4

New CAS Information Use Policies, enter HELP USAGETERMS for details.

TSCA INFORMATION NOW CURRENT THROUGH JULY 14, 2005

Please note that search-term pricing does apply when
conducting SmartSELECT searches.

*
* The CA roles and document type information have been removed from *
* the IDE default display format and the ED field has been added, *
* effective March 20, 2005. A new display format, IDERL, is now *
* available and contains the CA role and document type information. *
*

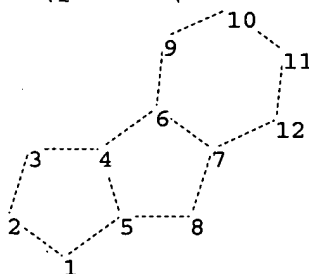
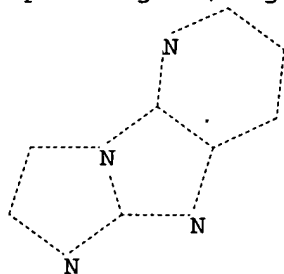
Structure search iteration limits have been increased. See HELP SLIMITS
for details.

REGISTRY includes numerically searchable data for experimental and
predicted properties as well as tags indicating availability of
experimental property data in the original document. For information
on property searching in REGISTRY, refer to:

<http://www.cas.org/ONLINE/UG/regprops.html>

=>

Uploading C:\Program Files\Stnexp\Queries\QUERIES\10771661.str



ring nodes :

1 2 3 4 5 6 7 8 9 10 11 12

ring bonds :

1-2 1-5 2-3 3-4 4-5 4-6 5-8 6-7 6-9 7-8 7-12 9-10 10-11 11-12

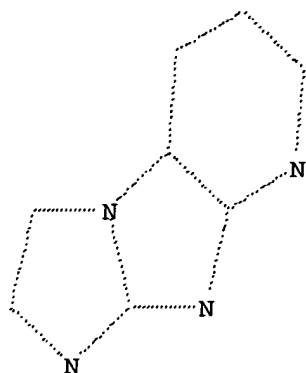
exact/norm bonds :

1-2 1-5 2-3 3-4 4-5 4-6 5-8 6-7 6-9 7-8 7-12 9-10 10-11 11-12

Match level :

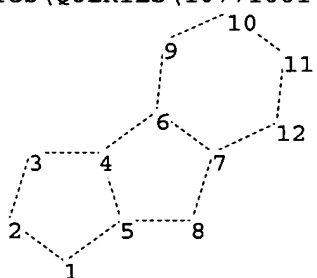
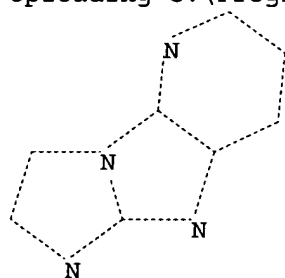
1:Atom 2:Atom 3:Atom 4:Atom 5:Atom 6:Atom 7:Atom 8:Atom 9:Atom 10:Atom
11:Atom 12:Atom

=> d
L1 HAS NO ANSWERS
L1 STR



Structure attributes must be viewed using STN Express query preparation.

=>
Uploading C:\Program Files\Stnexp\Queries\QUERIES\10771661.str

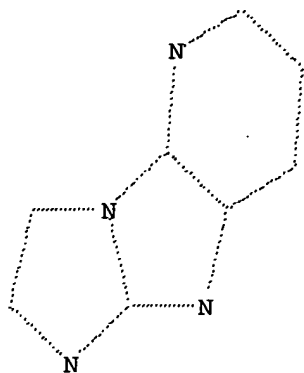


ring nodes :
1 2 3 4 5 6 7 8 9 10 11 12
ring bonds :
1-2 1-5 2-3 3-4 4-5 4-6 5-8 6-7 6-9 7-8 7-12 9-10 10-11 11-12
exact/norm bonds :
1-2 1-5 2-3 3-4 4-5 4-6 5-8 6-7 6-9 7-8 7-12 9-10 10-11 11-12

Match level :
1:Atom 2:Atom 3:Atom 4:Atom 5:Atom 6:Atom 7:Atom 8:Atom 9:Atom 10:Atom
11:Atom 12:Atom

L2 STRUCTURE UPLOADED

=> d
L2 HAS NO ANSWERS
L2 STR



Structure attributes must be viewed using STN Express query preparation.

=> s l1

SAMPLE SEARCH INITIATED 06:34:41 FILE 'REGISTRY'
SAMPLE SCREEN SEARCH COMPLETED - 35 TO ITERATE

100.0% PROCESSED 35 ITERATIONS 3 ANSWERS
SEARCH TIME: 00.00.01

FULL FILE PROJECTIONS: ONLINE **COMPLETE**
BATCH **COMPLETE**
PROJECTED ITERATIONS: 346 TO 1054
PROJECTED ANSWERS: 3 TO 163

L3 3 SEA SSS SAM L1

=> s l1 full

FULL SEARCH INITIATED 06:34:44 FILE 'REGISTRY'
FULL SCREEN SEARCH COMPLETED - 764 TO ITERATE

100.0% PROCESSED 764 ITERATIONS 90 ANSWERS
SEARCH TIME: 00.00.01

L4 90 SEA SSS FUL L1

=> s l2

SAMPLE SEARCH INITIATED 06:34:46 FILE 'REGISTRY'
SAMPLE SCREEN SEARCH COMPLETED - 35 TO ITERATE

100.0% PROCESSED 35 ITERATIONS 0 ANSWERS
SEARCH TIME: 00.00.01

FULL FILE PROJECTIONS: ONLINE **COMPLETE**
BATCH **COMPLETE**
PROJECTED ITERATIONS: 346 TO 1054
PROJECTED ANSWERS: 0 TO 0

L5 0 SEA SSS SAM L2

=> s l2 full

FULL SEARCH INITIATED 06:34:50 FILE 'REGISTRY'
FULL SCREEN SEARCH COMPLETED - 764 TO ITERATE

100.0% PROCESSED 764 ITERATIONS 8 ANSWERS
SEARCH TIME: 00.00.01

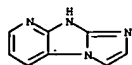
L6 8 SEA SSS FUL L2

```
=> s l4 and caplus/lc
      48645232 CAPLUS/LC
L7      87 L4 AND CAPLUS/LC
```

```
=> s l4 not l7
L8      3 L4 NOT L7
```

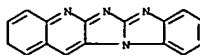
```
=> d l8 1-3
```

L8 ANSWER 1 OF 3 REGISTRY COPYRIGHT 2005 ACS on STN
 RN 444725-65-9 REGISTRY
 ED Entered STN: 22 Aug 2002
 CN 1H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine (9CI) (CA INDEX NAME)
 FS 3D CONCORD
 MF C9 H6 N4
 CI RPS
 SR CA Index Guide or Ring Systems Handbook



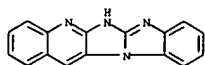
PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

L8 ANSWER 2 OF 3 REGISTRY COPYRIGHT 2005 ACS on STN
 RN 50288-09-0 REGISTRY
 ED Entered STN: 16 Nov 1984
 CN 2H-Benzimidazo[1',2':1,2]imidazo[4,5-b]quinoline (9CI) (CA INDEX NAME)
 FS 3D CONCORD
 MF C16 H10 N4
 CI RPS



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

L8 ANSWER 3 OF 3 REGISTRY COPYRIGHT 2005 ACS on STN
 RN 50287-77-9 REGISTRY
 ED Entered STN: 16 Nov 1984
 CN 7H-Benzimidazo[1',2':1,2]imidazo[4,5-b]quinoline (9CI) (CA INDEX NAME)
 FS 3D CONCORD
 MF C16 H10 N4



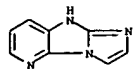
PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

=> s 16 and caplus/lc
48645232 CAPLUS/LC
L9 7 L6 AND CAPLUS/LC

=> s 16 not 19
L10 1 L6 NOT L9

=> d 110

L10 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2005 ACS on STN
RN 502422-99-3 REGISTRY
ED Entered STN: 09 Apr 2003
CN 1H-Imidazo[2',1':2,3]imidazo[4,5-b]pyridine (9CI) (CA INDEX NAME)
FS 3D CONCORD
MF C8 H6 N4
CI RPS
SR CA Index Guide or Ring Systems Handbook
LC STN Files: CHEMCATS



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

=> fil chemcats
COST IN U.S. DOLLARS

SINCE FILE	TOTAL
ENTRY	SESSION
340.08	340.29

FULL ESTIMATED COST

FILE 'CHEMCATS' ENTERED AT 06:36:10 ON 01 NOV 2005
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2005 American Chemical Society (ACS)

FILE LAST UPDATED 29 OCTOBER 2005 (20051029/UP)

For details on recent updates in CHEMCATS, enter NEWS FILE at an arrow prompt. For the list of suppliers currently in the file, enter HELP SPA, HELP SPBC, HELP SPDH, HELP SPIN, HELP SPOP, and HELP SPQZ. For the list of current catalogs, enter HELP CTA, HELP CTBC, HELP CTDH, HELP CTIN, HELP CTOP, and HELP CTQZ.

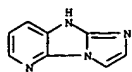
This database is provided on an "as is" basis. Please consult the suppliers for current information regarding pricing, regional availability, available quantities, purities, etc. THERE ARE NO WARRANTIES OF ANY KIND, EITHER EXPRESSED OR IMPLIED. ACS is not liable for any loss of profit, goodwill or any other damages arising out of the use of this database..

CHEMCATS now contains more than 8 million records. See HELP CONTENT and NEWS FILE for details.

=> s l10
L11 1 L10

=> d l11

L11 ANSWER 1 OF 1 CHEMCATS COPYRIGHT 2005 ACS on STN
Accession No. (AN): 2005:209447 CHEMCATS
Catalog Name (CO): Chemstep Product List
Publication Date (PD): 28 Feb 2005
Order Number (ON): 42453
Chemical Name (CN): 1H-Imidazo[2,1:2,3]imidazo[4,5-b]pyridine
CAS Registry No. (RN): 502422-99-3
Structure :



=> fil caplus
COST IN U.S. DOLLARS

SINCE FILE	TOTAL
ENTRY	SESSION
2.74	343.03

FULL ESTIMATED COST

FILE 'CAPLUS' ENTERED AT 06:36:24 ON 01 NOV 2005
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2005 AMERICAN CHEMICAL SOCIETY (ACS)

Copyright of the articles to which records in this database refer is held by the publishers listed in the PUBLISHER (PB) field (available for records published or updated in Chemical Abstracts after December 26, 1996), unless otherwise indicated in the original publications. The CA Lexicon is the copyrighted intellectual property of the American Chemical Society and is provided to assist you in searching databases on STN. Any dissemination, distribution, copying, or storing of this information, without the prior written consent of CAS, is strictly prohibited.

FILE COVERS 1907 - 1 Nov 2005 VOL 143 ISS 19
FILE LAST UPDATED: 30 Oct 2005 (20051030/ED)

Effective October 17, 2005, revised CAS Information Use Policies apply. They are available for your review at:

<http://www.cas.org/infopolicy.html>

=> d his

(FILE 'HOME' ENTERED AT 06:33:54 ON 01 NOV 2005)

FILE 'REGISTRY' ENTERED AT 06:34:02 ON 01 NOV 2005

L1	STRUCTURE UPLOADED
L2	STRUCTURE UPLOADED
L3	3 S L1
L4	90 S L1 FULL
L5	0 S L2
L6	8 S L2 FULL
L7	87 S L4 AND CAPLUS/LC
L8	3 S L4 NOT L7
L9	7 S L6 AND CAPLUS/LC
L10	1 S L6 NOT L9

FILE 'CHEMCATS' ENTERED AT 06:36:10 ON 01 NOV 2005

L11	1 S L10
-----	---------

FILE 'CAPLUS' ENTERED AT 06:36:24 ON 01 NOV 2005

=> s 17

L12	2 L7
-----	------

=> s 19

L13	1 L9
-----	------

=> s 19 not 17

	1 L9
	2 L7
L14	1 L9 NOT L7

=> d ibib abs hitstr l12 1-2

ACCESSION NUMBER: 2002:574934 CAPLUS

DOCUMENT NUMBER: 137:140524

TITLE: Preparation of imidazo fused heterocycles as corticotropin releasing factor inhibitors
 INVENTOR(S): Dubowchik, Gene M.; Han, Xiaojun; Vrduhala, Vivekananda M.; Zuev, Dmitry; Dasgupta, Bireshwar; Michne, Jodi A.

PATENT ASSIGNEE(S): Bristol-Myers Squibb Company, USA
 SOURCE: PCT Int. Appl., 321 pp.
 CODEN: PIXOXD2

DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002058704	A1	20020801	WO 2002-US841	20020111
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, GR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LA, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MY, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2434558	AA	20020801	CA 2002-2434558	20020111
US 2002183375	A1	20021205	US 2002-44183	20020111
US 6888004	B2	20050503		
EP 1359916	A1	20031112	EP 2002-705754	20020111
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
EE 200300342	A	20031215	EE 2003-342	20020111
BR 2002006698	A	20040420	BR 2002-6698	20020111
CN 1499972	A	20040526	CN 2002-807135	20020111
JP 2004531475	T2	20041014	JP 2002-559038	20020111
ZA 2003005531	A	20040727	ZA 2003-5531	20030717
BG 107999	A	20040831	BG 2003-107999	20030717
NO 2003003350	A	20030922	NO 2003-3350	20030725
US 2004254382	A1	20041216	US 2004-767645	20040129
US 200425130	A1	20041111	US 2004-771661	20040204
US 2004225001	A1	20041111	US 2004-771766	20040204
US 2004235924	A1	20041125	US 2004-772027	20040204
PRIORITY APPLN. INFO.: US 2001-264570P P 20010126				
US 2002-44183 A3 20020111				
WO 2002-US841 W 20020111				

OTHER SOURCE(S): MARPAT 137:140524
 GI

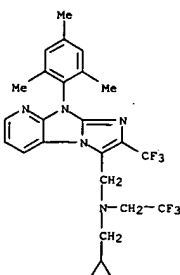
444335-96-0P

RL: PAC (Pharmacological activity); SPN (Synthetic preparation); THU (Therapeutic use); BIOL. (Biological study); PREP (Preparation); USES (Uses)

(prepn. of imidazo fused heterocycles as corticotropin releasing factor inhibitors)

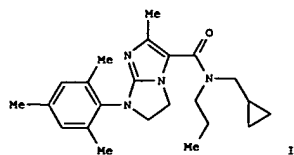
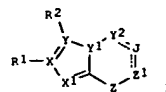
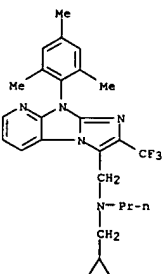
RN 444324-03-2 CAPLUS

CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, N-(cyclopropylmethyl)-N-(2,2,2-trifluoroethyl)-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



RN 444324-04-3 CAPLUS

CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, N-(cyclopropylmethyl)-N-propyl-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



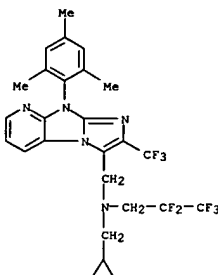
AB The title compds. [I; R1 = H, alkyl, haloalkyl, etc.; R2 = CDNR3R4, CH2NR3R4, etc.; D = O, S; R3, R4 = H, alkyl, haloalkyl, etc.; or NR3R4 = 5-6 membered heterocycle; X = C; Y = C; X1 = N; Y1 = N; Y2 = N, CH, CH2, CO, etc.; J = a bond, CH, CH2, CO, etc.; Z1 = CH, CH2, CO, etc.; Z = NV (wherein V = (un)substituted Ph, 2- or 3-pyridyl)], useful for the treatment of depression, anxiety, affective disorders, feeding disorders, post-traumatic stress disorder, headache, drug addiction, inflammatory disorders, drug or alc. withdrawal symptoms and other conditions the treatment of which can be effected by the antagonism of the CRF-1 receptor, were prepared E.g., a 5-step synthesis of II (starting with 2,4,6-trimethylaniline) which showed Ki of < 1,000 nM against CRF1 receptor binding.

IT 444324-03-2P 444324-04-3P 444324-05-4P
 444324-06-5P 444324-07-6P 444324-08-7P
 444324-09-8P 444324-10-9P 444324-11-2P
 444324-12-3P 444324-13-4P 444324-14-5P
 444324-15-6P 444324-16-7P 444324-17-8P
 444324-18-9P 444324-19-0P 444324-20-3P
 444324-21-4P 444324-22-5P 444324-23-6P
 444324-24-7P 444324-25-8P 444324-26-9P
 444324-27-0P 444324-28-1P 444324-29-2P
 444324-30-3P 444324-31-6P 444324-32-7P
 444324-33-8P 444324-34-9P 444324-35-0P
 444324-36-1P 444324-37-2P 444324-38-3P
 444324-39-4P 444324-40-7P 444324-41-8P
 444324-42-9P 444324-43-0P 444324-44-1P
 444324-45-2P 444324-46-3P 444324-47-4P
 444324-48-5P 444324-49-6P 444324-50-9P
 444324-51-0P 444324-52-1P 444324-53-2P
 444324-54-3P 444324-55-4P 444324-56-5P
 444324-57-6P 444324-58-7P 444324-59-8P
 444324-60-1P 444324-61-2P 444324-62-3P
 444324-63-4P 444324-64-5P 444324-65-6P
 444324-66-7P 444324-67-8P 444324-68-9P
 444324-69-0P 444324-70-3P 444324-71-4P
 444324-72-5P 444324-73-6P 444324-74-7P

RN 444324-05-4 CAPLUS

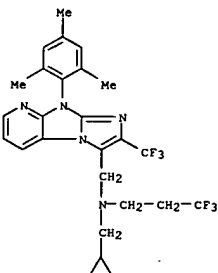
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,

N-(cyclopropylmethyl)-N-(2,2,3,3,3-pentafluoropropyl)-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



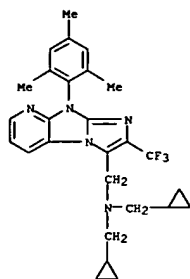
RN 444324-06-5 CAPLUS

CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, N-(cyclopropylmethyl)-2-(trifluoromethyl)-N-(3,3,3-trifluoropropyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)

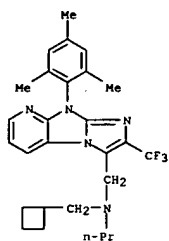


RN 444324-07-6 CAPLUS

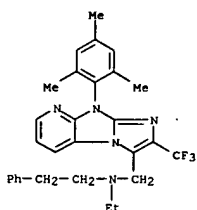
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, N,N-bis(cyclopropylmethyl)-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



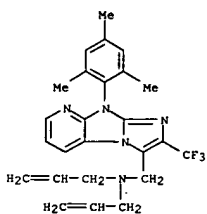
RN 444324-08-7 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N-(cyclobutylmethyl)-N-propyl-2-(trifluoromethyl)-9-(2,4,6-
trimethylphenyl)- (9CI) (CA INDEX NAME)



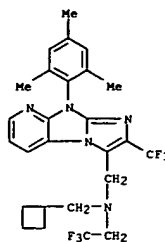
RN 444324-09-8 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N-(cyclobutylmethyl)-N-(2,2,2-trifluoroethyl)-2-(trifluoromethyl)-9-(2,4,6-
trimethylphenyl)- (9CI) (CA INDEX NAME)



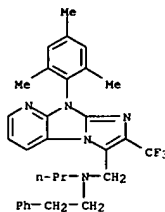
RN 444324-12-3 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N,N-di-2-propenyl-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI)
(CA INDEX NAME)



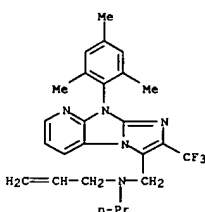
RN 444324-13-4 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N-2-propenyl-N-propyl-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)-
(9CI) (CA INDEX NAME)



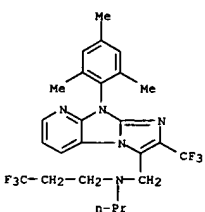
RN 444324-10-1 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N-(2-phenylethyl)-N-propyl-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)-
(9CI) (CA INDEX NAME)



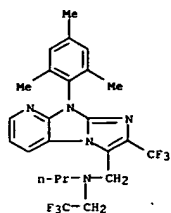
RN 444324-11-2 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N-ethyl-N-(2-phenylethyl)-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)-
(9CI) (CA INDEX NAME)



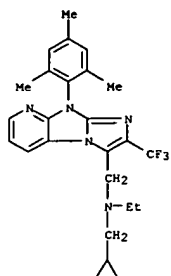
RN 444324-14-5 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N-propyl-2-(trifluoromethyl)-N-(3,3,3-trifluoropropyl)-9-(2,4,6-
trimethylphenyl)- (9CI) (CA INDEX NAME)



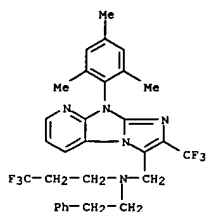
RN 444324-15-6 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N-propyl-N-(2,2,2-trifluoroethyl)-2-(trifluoromethyl)-9-(2,4,6-
trimethylphenyl)- (9CI) (CA INDEX NAME)



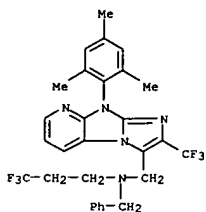
RN 444324-16-7 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, N-(cyclopropylmethyl)-N-ethyl-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



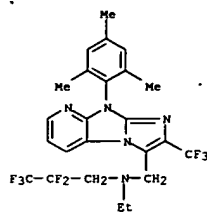
RN 444324-17-8 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, N-ethyl-N-(2,2,3,3,3-pentafluoropropyl)-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



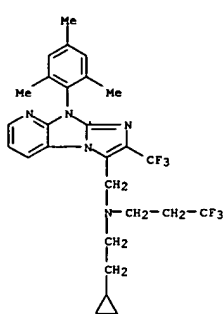
RN 444324-20-3 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, N-(phenylmethyl)-2-(trifluoromethyl)-N-(3,3,3-trifluoropropyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



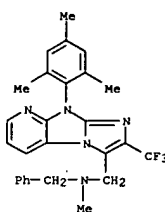
RN 444324-21-4 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, N-methyl-N-(phenylmethyl)-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



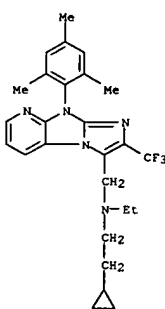
RN 444324-18-9 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, N-(2-cyclopropylethyl)-2-(trifluoromethyl)-N-(3,3,3-trifluoropropyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



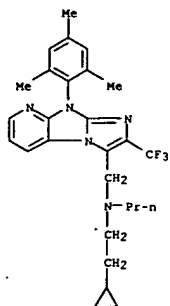
RN 444324-19-0 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, N-(2-phenylethyl)-2-(trifluoromethyl)-N-(3,3,3-trifluoropropyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



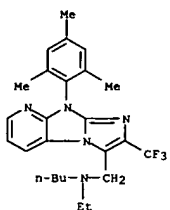
RN 444324-22-5 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, N-(2-cyclopropylethyl)-N-ethyl-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



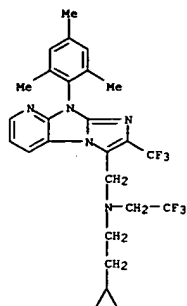
RN 444324-23-6 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, N-(2-cyclopropylethyl)-N-propyl-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



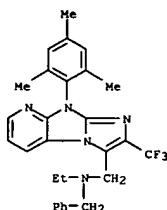
RN 444324-24-7 CAPLUS
 CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
 N-butyl-N-ethyl-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



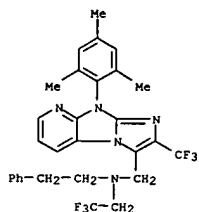
RN 444324-25-8 CAPLUS
 CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
 N-(2-cyclopropylethyl)-N-(2,2,2-trifluoroethyl)-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



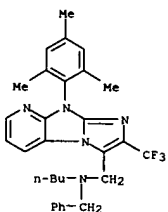
RN 444324-26-9 CAPLUS
 CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
 N-ethyl-N-(phenylmethyl)-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



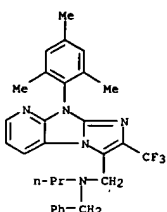
RN 444324-27-0 CAPLUS
 CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
 N-(2-phenylethyl)-N-(2,2,2-trifluoroethyl)-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



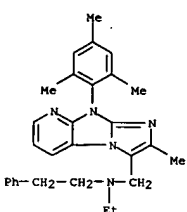
RN 444324-28-1 CAPLUS
 CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
 N-butyl-N-(phenylmethyl)-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



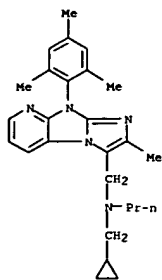
RN 444324-29-2 CAPLUS
 CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
 N-(phenylmethyl)-N-propyl-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



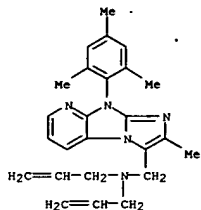
RN 444324-30-5 CAPLUS
 CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
 N-ethyl-N-(2-phenylethyl)-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



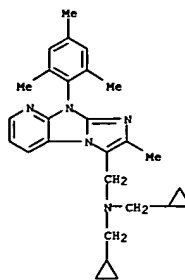
RN 444324-31-6 CAPLUS
 CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
 N-(cyclopropylmethyl)-2-methyl-N-propyl-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



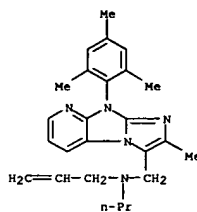
RN 444324-32-7 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
2-methyl-N,N-di-2-propenyl-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



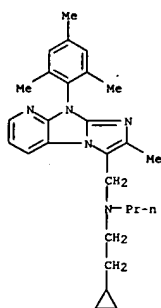
RN 444324-33-8 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N,N-bis(cyclopropylmethyl)-2-methyl-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



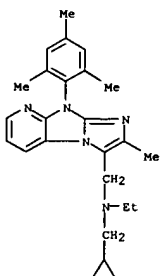
RN 444324-34-9 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
2-methyl-N-2-propenyl-N-propyl-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



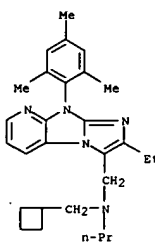
RN 444324-35-0 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N-(2-cyclopropylethyl)-2-methyl-N-propyl-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



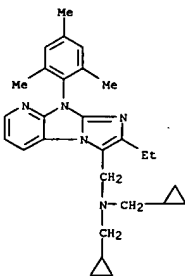
RN 444324-36-1 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N-(cyclopropylmethyl)-N-ethyl-2-methyl-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



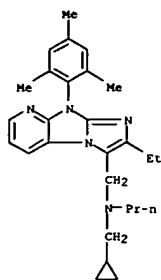
RN 444324-37-2 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N-(cyclobutylmethyl)-2-ethyl-N-propyl-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



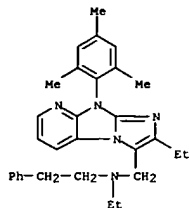
RN 444324-38-3 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N,N-bis(cyclopropylmethyl)-2-ethyl-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



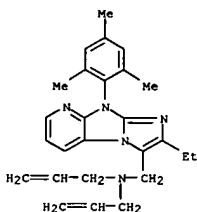
RN 444324-39-4 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N-(cyclopropylmethyl)-2-ethyl-N-propyl-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



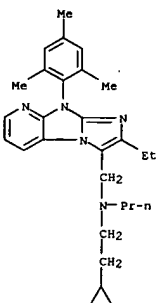
RN 444324-40-7 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N-(2-cyclopropylethyl)-N-(2-phenylethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



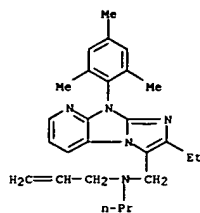
RN 444324-41-8 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
2-ethyl-N-(2-propenyl)-N-propyl-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



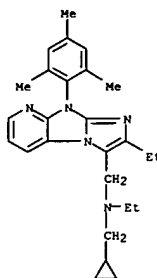
RN 444324-44-1 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N-(2-cyclopropylethyl)-2-ethyl-N-propyl-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



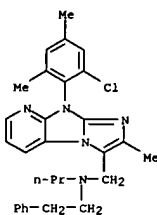
RN 444324-45-2 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
9-(2-chloro-4,6-dimethylphenyl)-2-methyl-N-(2-phenylethyl)-N-propyl- (9CI) (CA INDEX NAME)



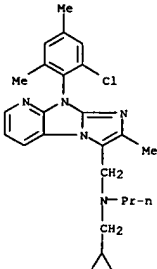
RN 444324-42-9 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N-(cyclopropylmethyl)-N,2-diethyl-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



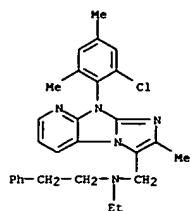
RN 444324-43-0 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
2-ethyl-N,N-di-2-propenyl-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



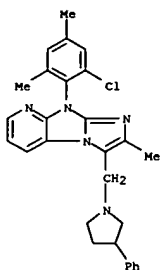
RN 444324-46-3 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
9-(2-chloro-4,6-dimethylphenyl)-N-(cyclopropylmethyl)-2-methyl-N-propyl- (9CI) (CA INDEX NAME)



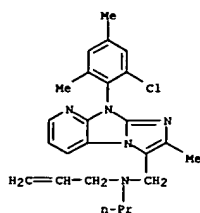
RN 444324-47-4 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
9-(2-chloro-4,6-dimethylphenyl)-N-ethyl-2-methyl-N-(2-phenylethyl)- (9CI) (CA INDEX NAME)



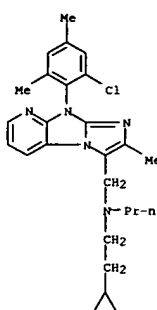
RN 444324-48-5 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine, 9-(2-chloro-4,6-dimethylphenyl)-2-methyl-3-[(3-phenyl-1-pyrrolidinyl)methyl]- (9CI) (CA INDEX NAME)



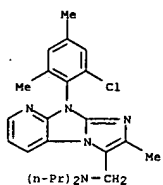
RN 444324-49-6 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, 9-(2-chloro-4,6-dimethylphenyl)-2-methyl-N-2-propenyl-N-propyl- (9CI) (CA INDEX NAME)



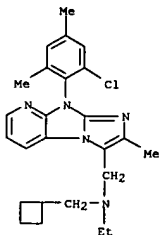
RN 444324-50-9 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, 9-(2-chloro-4,6-dimethylphenyl)-N-(2-cyclopropylethyl)-2-methyl-N-propyl- (9CI) (CA INDEX NAME)



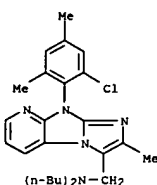
RN 444324-51-0 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, 9-(2-chloro-4,6-dimethylphenyl)-2-methyl-N,N-dipropyl- (9CI) (CA INDEX NAME)



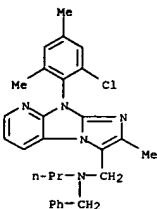
RN 444324-52-1 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, 9-(2-chloro-4,6-dimethylphenyl)-N-(cyclobutylmethyl)-N-ethyl-2-methyl- (9CI) (CA INDEX NAME)



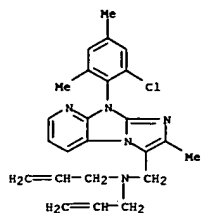
RN 444324-53-2 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, N,N-dibutyl-9-(2-chloro-4,6-dimethylphenyl)-2-methyl- (9CI) (CA INDEX NAME)



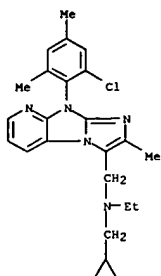
RN 444324-54-3 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, 9-(2-chloro-4,6-dimethylphenyl)-2-methyl-N-(phenylmethyl)-N-propyl- (9CI) (CA INDEX NAME)



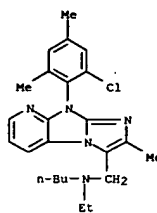
RN 444324-55-4 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine, 9-(2-chloro-4,6-dimethylphenyl)-2-methyl-N,N-di-2-propenyl- (9CI) (CA INDEX NAME)



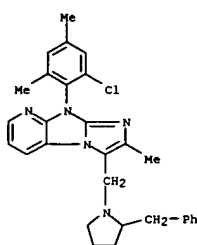
RN 444324-56-5 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
9-(2-chloro-4,6-dimethylphenyl)-N-(cyclopropylmethyl)-N-ethyl-2-methyl-
(9CI) (CA INDEX NAME)



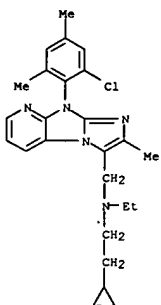
RN 444324-57-6 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N-butyl-9-(2-chloro-4,6-dimethylphenyl)-N-ethyl-2-methyl- (9CI) (CA
INDEX NAME)



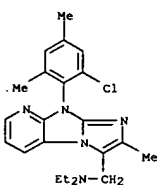
RN 444324-58-7 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine, 9-(2-chloro-4,6-
dimethylphenyl)-2-methyl-3-[(2-phenylmethyl)-1-pyrrolidinyl]methyl-
(9CI) (CA INDEX NAME)



RN 444324-59-8 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
9-(2-chloro-4,6-dimethylphenyl)-N-(2-cyclopropylethyl)-N-ethyl-2-methyl-
(9CI) (CA INDEX NAME)

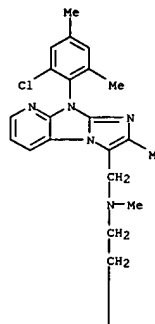


RN 444324-60-1 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
9-(2-chloro-4,6-dimethylphenyl)-N,N-diethyl-2-methyl- (9CI) (CA INDEX
NAME)



RN 444324-61-2 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
9-(2-chloro-4,6-dimethylphenyl)-N,2-dimethyl-N-[2-(3-pyridinyl)ethyl]-
(9CI) (CA INDEX NAME)

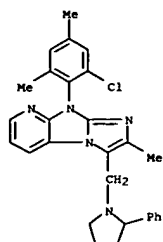
PAGE 1-A



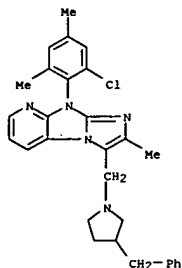
PAGE 2-A



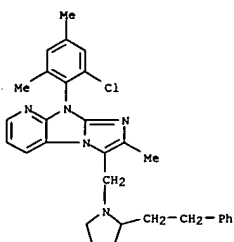
RN 444324-62-3 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine, 9-(2-chloro-4,6-
dimethylphenyl)-2-methyl-3-[(2-phenyl-1-pyrrolidinyl)methyl]- (9CI) (CA
INDEX NAME)



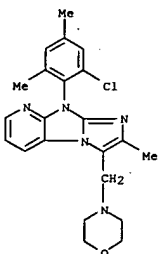
RN 444324-63-4 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine, 9-(2-chloro-4,6-dimethylphenyl)-2-methyl-3-[(3-(phenylmethyl)-1-pyrrolidinyl)methyl]- (9CI) (CA INDEX NAME)



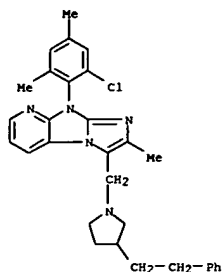
RN 444324-64-5 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine, 9-(2-chloro-4,6-dimethylphenyl)-2-methyl-3-[(3-(2-phenylethyl)-1-pyrrolidinyl)methyl]- (9CI) (CA INDEX NAME)



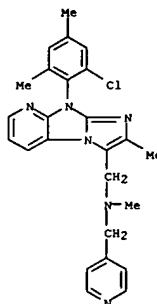
RN 444324-67-8 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine, 9-(2-chloro-4,6-dimethylphenyl)-2-methyl-3-[(4-morpholinylmethyl)- (9CI) (CA INDEX NAME)



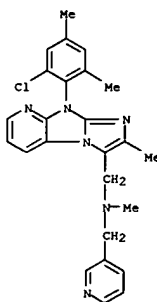
RN 444324-68-9 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine, 9-(2-chloro-4,6-dimethylphenyl)-N,2-dimethyl-N-(3-pyridinylmethyl)- (9CI) (CA INDEX NAME)



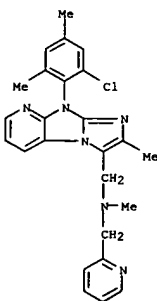
RN 444324-65-6 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine, 9-(2-chloro-4,6-dimethylphenyl)-N,2-dimethyl-N-(4-pyridinylmethyl)- (9CI) (CA INDEX NAME)



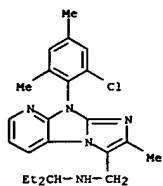
RN 444324-66-7 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine, 9-(2-chloro-4,6-dimethylphenyl)-N,2-dimethyl-N-(2-pyridinylmethyl)- (9CI) (CA INDEX NAME)



RN 444324-69-0 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine, 9-(2-chloro-4,6-dimethylphenyl)-N,2-dimethyl-N-(2-pyridinylmethyl)- (9CI) (CA INDEX NAME)

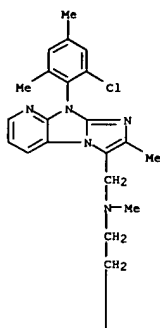


RN 444324-70-3 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine, 9-(2-chloro-4,6-dimethylphenyl)-N,2-dimethyl-N-(1-ethylpropyl)-2-methyl- (9CI) (CA INDEX NAME)

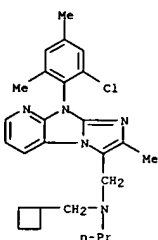


RN 444324-71-4 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
9-(2-chloro-4,6-dimethylphenyl)-N,2-dimethyl-N-[2-(4-pyridinyl)ethyl]-
(9CI) (CA INDEX NAME)

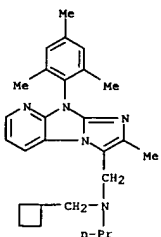
PAGE 1-A



PAGE 2-A



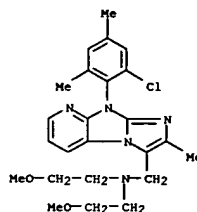
RN 444335-96-0 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
N-(cyclobutylmethyl)-2-methyl-N-propyl-9-(2,4,6-trimethylphenyl)-
(9CI) (CA INDEX NAME)



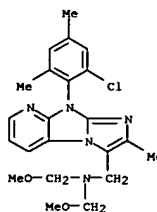
IT 444326-07-2P 444326-08-3P 444326-09-4P
444326-10-7P 444326-11-8P 444326-14-1P
444326-15-2P 444326-16-3P 444326-17-4P
444326-18-5P 444326-20-9P 444326-21-0P
444326-22-1P
RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation); RACT
(Reactant or reagent)
(preparation of imidazo fused heterocycles as corticotropin releasing
factor
inhibitors)

RN 444326-07-2 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-carboxylic acid,
2-methyl-9-(2,4,6-trimethylphenyl)-, ethyl ester (9CI) (CA INDEX NAME)

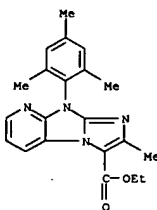
RN 444324-72-5 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
9-(2-chloro-4,6-dimethylphenyl)-N,N-bis(2-methoxyethyl)-2-methyl-
(9CI) (CA INDEX NAME)



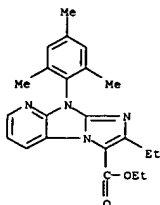
RN 444324-73-6 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
9-(2-chloro-4,6-dimethylphenyl)-N,N-bis(methoxymethyl)-2-methyl-
(9CI) (CA INDEX NAME)



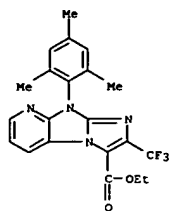
RN 444326-51-6 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanamine,
9-(2-chloro-4,6-dimethylphenyl)-N-(cyclobutylmethyl)-2-methyl-N-propyl-
(9CI) (CA INDEX NAME)



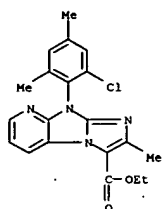
RN 444326-08-3 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-carboxylic acid,
2-ethyl-9-(2,4,6-trimethylphenyl)-, ethyl ester (9CI) (CA INDEX NAME)



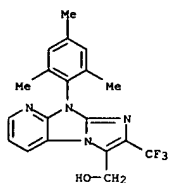
RN 444326-09-4 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-carboxylic acid,
2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)-, ethyl ester (9CI) (CA
INDEX NAME)



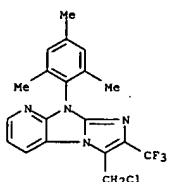
RN 444326-10-7 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-carboxylic acid, 9-(2-chloro-4,6-dimethylphenyl)-2-methyl-, ethyl ester (9CI) (CA INDEX NAME)



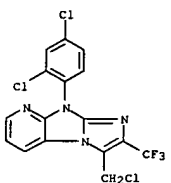
RN 444326-11-8 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanol, 2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



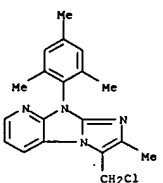
RN 444326-17-4 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine, 3-(chloromethyl)-2-(trifluoromethyl)-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



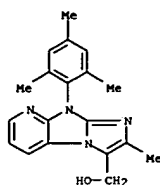
RN 444326-18-5 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine, 3-(chloromethyl)-9-(2,4-dichlorophenyl)-2-(trifluoromethyl)- (9CI) (CA INDEX NAME)



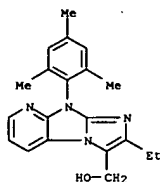
RN 444326-20-9 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine, 3-(chloromethyl)-2-methyl-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



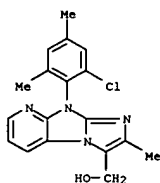
RN 444326-14-1 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanol, 2-methyl-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



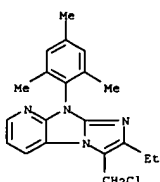
RN 444326-15-2 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanol, 2-ethyl-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



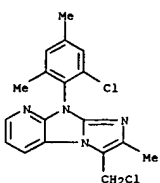
RN 444326-16-3 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine-3-methanol, 9-(2-chloro-4,6-dimethylphenyl)-2-methyl- (9CI) (CA INDEX NAME)



RN 444326-21-0 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine, 3-(chloromethyl)-2-ethyl-9-(2,4,6-trimethylphenyl)- (9CI) (CA INDEX NAME)



RN 444326-22-1 CAPLUS
CN 9H-Imidazo[1',2':1,2]imidazo[4,5-b]pyridine, 9-(2-chloro-4,6-dimethylphenyl)-3-(chloromethyl)-2-methyl- (9CI) (CA INDEX NAME)



REFERENCE COUNT: 1 THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1973:492104 CAPLUS

DOCUMENT NUMBER: 79:92104

TITLE: Imidazo[1,2-a]benzimidazole derivatives. IX.

Compounds of the 2-oxo-2,3-dihydroimidazo[1,2-

a]benzimidazole series and their transformations

Borisova, T. A.; Simonov, A. M.; Anisimova, V. A.

Rostov. Gos. Univ., Rostov, USSR

Khimiya Geterotsiklicheskikh Soedinenii (1973), (6),

803-6

CODEN: KGSSAQ; ISSN: 0132-6244

DOCUMENT TYPE: Journal

LANGUAGE: Russian

GI For diagram(s), see printed CA Issue.

AB Hydrolysis of imidazobenzimidazole (I; R = Me) by HCl yielded 92%

benzimidazole (II; X = NH.HCl), which was nitrosated by NaNO₂ to give 15%

II (X = NNO). Basic hydrolysis of I afforded the keto acid (II; X = O).

Oxidation of I by KMnO₄ gave azo derivative (III). 3-

Arylideneimidazobenzimidazoles (IV; X = p-O₂NC₆H₄CH,

5-nitro-2-furylidene, 5-nitro-2-furylidene,

o-O₂NC₆H₄CH; R = Me, PhCH₂) were prepared in 62-76% yields by

condensation

of I with the appropriate aldehyde.

IT 43182-01-0P

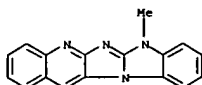
RL: SPN (Synthetic preparation); PREP (Preparation)

(Preparation of)

RN 43182-01-0 CAPLUS

CN 7H-Benzimidazo[1',2':1,2]imidazo[4,5-b]quinoline, 7-methyl- (9CI) (CA

INDEX NAME)



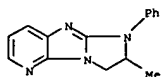
=> d ibib abs hitstr 113

ACCESSION NUMBER: 2002:805373 CAPLUS
 DOCUMENT NUMBER: 138:238142
 TITLE: Heterocyclization reaction of 2-(2-methylaziridin-1-yl)-3-ureidopyridines under Appel's conditions
 AUTHOR(S): Lim, Ji-Sun; Lee, Kee-Jung
 CORPORATE SOURCE: Department of Industrial Chemistry, Hanyang University, Seoul, 133-791, S. Korea
 SOURCE: Journal of Heterocyclic Chemistry (2002), 39(5), 975-980

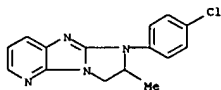
CODEN: JHCTAD; ISSN: 0022-152X
 PUBLISHER: HeteroCorporation
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 OTHER SOURCE(S): CASREACT 138:238142

AB The reaction of 2-(2-methylaziridin-1-yl)-3-ureidopyridines with triphenylphosphine, carbon tetrachloride, and triethylamine (Appel's conditions) led to the corresponding carbodiimides, which underwent intramol. cycloaddn. reaction with aziridine under the reaction conditions to give the pyridinefused heterocycles, 2,3-dihydro-1H-imidazo[2',1':2,3]imidazo[4,5-b]pyridines and 12,13-dihydro-5H-1,3-benzodiazepino[2',3':2,3]imidazo[4,5-b]pyridines.
 IT 501433-85-8P 501433-86-9P 501433-87-0P
 501433-88-1P 501433-89-2P 501433-90-5P
 501433-91-6P
 RL: SPN (Synthetic preparation); PREP (Preparation)
 (heterocyclization of (methylaziridinyl)ureidopyridines under Appel's conditions)

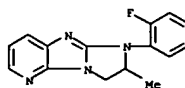
RN 501433-85-8 CAPLUS
 CN 1H-Imidazo[2',1':2,3]imidazo[4,5-b]pyridine, 2,3-dihydro-2-methyl-1-phenyl- (9CI) (CA INDEX NAME)



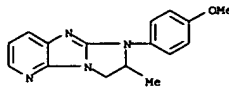
RN 501433-86-9 CAPLUS
 CN 1H-Imidazo[2',1':2,3]imidazo[4,5-b]pyridine, 1-(4-chlorophenyl)-2,3-dihydro-2-methyl- (9CI) (CA INDEX NAME)



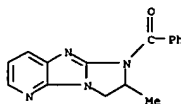
RN 501433-87-0 CAPLUS
 CN 1H-Imidazo[2',1':2,3]imidazo[4,5-b]pyridine, 1-(2-fluorophenyl)-2,3-dihydro-2-methyl- (9CI) (CA INDEX NAME)



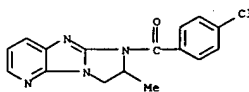
RN 501433-88-1 CAPLUS
 CN 1H-Imidazo[2',1':2,3]imidazo[4,5-b]pyridine, 2,3-dihydro-1-(4-methoxyphenyl)-2-methyl- (9CI) (CA INDEX NAME)



RN 501433-89-2 CAPLUS
 CN 1H-Imidazo[2',1':2,3]imidazo[4,5-b]pyridine, 1-benzoyl-2,3-dihydro-2-methyl- (9CI) (CA INDEX NAME)

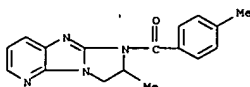


RN 501433-90-5 CAPLUS
 CN 1H-Imidazo[2',1':2,3]imidazo[4,5-b]pyridine, 1-(4-chlorobenzoyl)-2,3-dihydro-2-methyl- (9CI) (CA INDEX NAME)



RN 501433-91-6 CAPLUS
 CN 1H-Imidazo[2',1':2,3]imidazo[4,5-b]pyridine, 2,3-dihydro-2-methyl-1-(4-methylbenzoyl)- (9CI) (CA INDEX NAME)

REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE REFORMAT



=> log y

COST IN U.S. DOLLARS

SINCE FILE

TOTAL

ENTRY

SESSION

FULL ESTIMATED COST

17.07

360.10

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)

SINCE FILE

TOTAL

ENTRY

SESSION

CA SUBSCRIBER PRICE

-2.19

-2.19

STN INTERNATIONAL LOGOFF AT 06:39:31 ON 01 NOV 2005



Bristol-Myers Squibb Company

P.O. Box 4000
Princeton, NJ 08543-4000 U.S.A.

Fax

via email: rebecca.Anderson@USPTO.gov

To:	Examiner Rebecca L. Anderson	From:	Nancy Turover
Fax:	571-273-0696	Phone:	203-677-7668
Date:	November 1, 2005	Fax:	203-677-6900
Re:	CT-2666-DIV-4 10/771,661	Pages:	125 including cover page

Dear Examiner Anderson:

As per your telephone request, we attach copies of following articles cited on the IDS dated April 27, 2004, for the above-referenced application .

Sheet 1 of 3 [AR] Dunn and Berridge (30 pages)

[AS] Gulyas, et al (5 pages)

[AT] McCarthy, et al (27 pages)

Sheet 2 of 3 [AA] Holsboer, et al (34 pages)

[AB] Banki, et al (7 pages)

[AC] Zobel, et al (11 pages)

[AF] McCarthy, et al [10 pages]

Regards,

Nancy Turover

Potent, structurally constrained agonists and competitive antagonists of corticotropin-releasing factor

J. GULYAS, C. RIVIER, M. PERRIN, S. C. KOERBER, S. SUTTON, A. CORRIGAN, S. L. LAHRICHI, A. G. CRAIG, W. VALE, AND J. RIVIER*

The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037

Contributed by W. Vale. The Salk Institute, La Jolla, CA, July 20, 1995

ABSTRACT Predictive methods, physicochemical measurements, and structure activity relationship studies suggest that corticotropin-releasing factor (CRF; corticoliberin), its family members, and competitive antagonists (resulting from N-terminal deletions) usually assume an α -helical conformation when interacting with the CRF receptor(s). To test this hypothesis further, we have scanned the whole sequence of the CRF antagonist [D-Phe¹², Nle^{21,38}]r/hCRF-(12-41) (r/hCRF, rat/human CRF; Nle, norleucine) with an i -(i + 3) bridge consisting of the Glu-Xaa-Xaa-Lys scaffold. We have found astressin (cyclo(30-33) [D-Phe¹², Nle^{21,38}, Glu³⁰, Lys³³]r/hCRF-(12-41)) to be approximately 30 times more potent than [D-Phe¹², Nle^{21,38}]r/hCRF-(12-41), our present standard, and 300 times more potent than the corresponding linear analog in an *in vitro* pituitary cell culture assay. Astressin has low affinity for the CRF binding protein and high affinity (K_i = 2 nM) for the cloned pituitary receptor. Radioiodinated [D-¹²⁵I-Tyr¹²]astressin was found to be a reliable ligand for binding assays. *In vivo*, astressin is significantly more potent than any previously tested antagonist in reducing hypophyseal corticotropin (ACTH) secretion in stressed or adrenalectomized rats. The cyclo(30-33) [Ac-Pro⁴, D-Phe¹², Nle^{21,38}, Glu³⁰, Lys³³]r/hCRF-(4-41) agonist and its linear analog are nearly equipotent, while the antagonist astressin and its linear form vary greatly in their potencies. This suggests that the lactam cyclization reinstates a structural constraint in the antagonists that is normally induced by the N terminus of the agonist.

Corticotropin-releasing factor (CRF; corticoliberin) is a 41-residue peptide amide which stimulates the release of corticotropin (ACTH) (1, 2) and acts within the brain to mediate a wide range of stress responses (3). The actions of CRF are mediated through binding to CRF receptors, several of which have been characterized recently (4-10). These receptors, like those for growth hormone-releasing factor, calcitonin, and vasoactive intestinal peptide, are coupled via G proteins and have seven putative transmembrane domains. The actions of CRF can also be modulated by a 37-kDa CRF-binding protein (CRF-BP) (11). To probe the physiological role of CRF, we have developed competitive antagonists that are particularly potent when administered in the central nervous system; however, these same analogs bind pituitary receptors with lower affinity than does CRF, and their peripheral administration results in weak and short-lived effects *in vivo* (12). Synthetic CRF antagonists such as the α -helical CRF-(9-41) (compound 2, Table 1) or analogs of [D-Phe¹², Nle^{21,38}]r/hCRF-(12-41) (compound 1) (r/hCRF, rat/human CRF; Nle, norleucine) have been used to characterize the physiological roles of CRF. However, these antagonists have suffered from limited solubility and persistence of intrinsic activity, as well as weak potency at the hypophyseal site of action. These factors may have been responsible for significant heterogeneity in the

ensuing ACTH response to stressors. In fact, none of the antagonists reported so far is potent enough to warrant clinical investigation since parenteral administration is the only convenient delivery route in humans.

Several options, mostly directed at stabilizing a bioactive conformation in solution, have been tested to increase the potency of CRF antagonists. The most successful approaches, as judged by results with rat pituitary cells in culture, have been the introduction of α -helix-inducing residues within the sequence (13), or the introduction of C^α-methylated amino acids (12) which are known to induce α -helicity. These strategies led to the development of compounds such as α -helical CRF-(9-41), [D-Phe¹², Nle^{21,38}]hCRF-(12-41), and [D-Phe¹², Nle^{21,38}, C^α-MeLeu³⁷]hCRF-(12-41). These compounds had higher potencies and, in the latter case, a significantly increased duration of action *in vivo* (12). A recent NMR study of hCRF in 66% (vol/vol) aqueous trifluoroethanol identified a well-defined α -helix between residues 6 and 36, with an extended N terminus and a disordered C terminus (14). The first half of the α -helix was clearly amphipathic, as recognized earlier from model studies (15, 16). All experimental and theoretical data pointed to the fact that CRF-like molecules assume an α -helical conformation upon binding to their receptors; nevertheless, we were still faced with the challenge of devising and implementing strategies (conceptual as well as synthetic) that would allow us to lock the CRF structure in its bioactive conformation (hypothetically α -helical).

One means of achieving conformational stability is to create side-chain-to-side-chain covalent bonding that is not available to most biological systems. Salt bridges are known to stabilize tertiary structures in proteins, and examples can be found of i -(i + 3) and i -(i + 4) interactions (Glu or Asp to Lys or Arg or His). Such salt bridges can be replaced in some cases by a covalent amide bond forming a lactam ring in molecules like growth hormone-releasing factor (17, 18) and CRF (16) that have a propensity for α -helix formation. Alternatively, the introduction of D-amino acids has been shown to stabilize turns. [D-Glu²⁰]oCRF (oCRF, ovine CRF) being significantly more potent than oCRF itself suggested the intriguing possibility of the presence of a turn in the middle of the CRF molecule. On the other hand, the presence of a glutamic acid residue at position 20 and a lysine residue at position 23 of oCRF was suggestive of a possible salt bridge stabilizing an α -helix (Glu is found in all characterized CRFs at position 20 while Lys is found at position 23 in ovine/caprine/bovine CRF and Arg is found at position 23 in rat/human/porcine/suckerfish CRF). A systematic study which considered bridge

Abbreviations: CZE, capillary zone electrophoresis; DCM, dichloromethane; DMF, dimethylformamide; Fmoc, 9-fluorenylmethoxycarbonyl; HBTU, O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate; LSIMS, liquid secondary ion mass spectrometry; Ofm, O-fluorenylmethyl; TBTU, O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid; CRF, corticotropin-releasing factor; r/h/o CRF, rat/human/ovine CRF; Nle, norleucine; CRF-BP, CRF-binding protein; ACTH, corticotropin; Boc, *tert*-butoxycarbonyl.

*To whom reprint requests should be addressed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

length, chirality, and positioning of the lactam bond led to the identification of cyclo(20-23)[D-Phe¹²,Glu²⁰,Lys²³,Nle^{21,28}]-hCRF-(12-41) as the most potent diastereomer (2.9 times the potency of compound 1). From these observations, we concluded that an *i*-(*i* + 3) Glu to Lys lactam bridge was compatible with α -helix stabilization and most likely was the best possible bridging moiety to fulfill our goal. To further test this hypothesis, we completed the synthesis of an *i*-(*i* + 3) bridge scan Glu-Xaa-Xaa-Lys of compound 1, which is one of the most potent CRF antagonists reported to date (12).

MATERIALS AND METHODS

Synthesis of CRF Analogs. All analogs shown in Table 1 were synthesized either manually or on a Beckman model 990 peptide synthesizer by using the solid-phase approach, a 4-methylbenzhydrylamine resin (16) and the *tert*-butoxycarbonyl (Boc) strategy with orthogonal protection [9-fluorenylmethoxycarbonyl (Fmoc) and *O*-fluorenylmethyl (Ofm)] of the side chains of residues to be cyclized (17). Amino acid derivatives Boc-Ala, Boc-Arg(Tos), Boc-Asn(Xan), Boc-Asp(cHex), Boc-Gln(Xan), Boc-Glu(cHex), Boc-His(Tos), Boc-Ile, Boc-Met, Boc-Leu, Boc-Phe, Boc-Pro, Boc-Ser(Bzl), Boc-Thr(Bzl), Boc-Tyr(2,6-Br₂-Bzl), and Boc-Val were obtained from Bachem. Boc-Glu(Ofm) and Boc-Lys(Fmoc) were synthesized as described (19). All solvents were reagent grade or better. The boc group was removed with 60% (vol/vol) trifluoroacetic acid (TFA) in dichloromethane (DCM) and 10% (vol/vol) ethanedithiol. Main-chain assembly was mediated by diisopropylcarbodiimide. Coupling time was 90–120 min followed by acetylation (excess acetic anhydride in DCM for 15 min). Three-fold excess protected amino acid was used based on the original substitution of the 4-methylbenzhydrylamine resin. Deprotection of the Fmoc group was achieved by using a solution of 20% (vol/vol) piperidine/80% dimethylformamide (DMF) (2 \times 10 min) followed by sequential washes with DMF, MeOH, 10% (vol/vol) triethylamine/DCM and DCM. Lactam formation was mediated by using *O*-(benzotriazol-1-yl)-*N,N,N'*-tetramethyluronium tetrafluoroborate (TBTU) or *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) in DMF or *N*-methylpyrrolidone. Best results were obtained when the peptide chain was assembled in its entirety prior to cleavage of the Fmoc- and Ofm-protecting groups and cyclization as shown (16). The peptides were cleaved and deprotected in HF and purified by using reversed-phase HPLC and two or three solvent systems (triethylammonium phosphate at pH 2.25 and/or 6.5 and 0.1% TFA) (20, 21).

Characterization of CRF Analogs. Peptides were characterized as shown in Table 1. Analogs were >90% pure by independent HPLC and capillary zone electrophoresis (CZE) criteria. Amino acid analyses gave the expected ratios (data not shown).

CZE. CZE was done by using a Beckman model P/ACE System 2000 controlled by an IBM Personal System/2 Model 50Z and by using a ChromJet integrator. Electrophoresis was performed in 0.1 M sodium phosphate (pH 2.5) except for α -helical CRF-(9–41), which was measured in 0.1 M sodium borate (pH 8.5).

Mass Spectroscopy. Liquid secondary ion mass spectrometry (LSIMS) mass spectra were measured with a JEOL model JMS-HX110 double-focusing mass spectrometer fitted with a Cs⁺ gun. An accelerating voltage of 10 kV and Cs⁺ gun voltage between 25 and 30 kV were employed; for further details, see ref. 16. Calculated values for protonated molecular ions were in agreement with those obtained when using LSIMS.

Optical Rotations. Optical rotations of peptides were measured (sodium D line) in 1% acetic acid (*c* = 1.0) by using a Perkin-Elmer model 241 polarimeter and a 100- μ l cell (16). Compound 8 was measured in 9% acetic acid.

Circular Dichroism (CD) Spectra (see Fig. 1). CD spectra were obtained with a computer-controlled Aviv Associates (Lakewood, NJ) model 62DS spectropolarimeter under the control of the manufacturer's 60DS operating system. Spectra were collected at 1.0-nm intervals in the range of 190–250 nm as the average of four runs using a 2.0-sec integration time and a spectral bandwidth of 2.0 nm in 0.5-mm cuvettes thermostated at 20°C. Spectra were collected under two sets of buffer conditions: 0.02 M sodium phosphate, pH 6.5, and the same buffer diluted 1:1 (vol/vol) with 2,2,2-trifluoroethanol. Concentrations were determined on the basis of the calculated molecular mass of the TFA salt of the purified lyophilized peptide. Residue molar ellipticities were calculated on the basis of the number of residues in each analog, irrespective of any side-chain bridging amide bonds.

In Vitro Pituitary Cell Culture Assay. CRF agonists were tested in an *in vitro* assay measuring stimulation of ACTH release by rat anterior pituitary cells in culture when using r/hCRF as the standard compound (2). CRF antagonists were tested in an *in vitro* assay measuring alteration of CRF-induced release of ACTH by rat anterior pituitary cells in culture when using [D-Phe¹²,Nle^{21,28}]h/rCRF-(12–41) as the standard compound (1, 2, 13). Each value represents the mean \pm SEM of triplicate wells. Relative potencies are shown with 95% confidence limits in parentheses. Most compounds were tested three times, yielding comparable potencies in these assays. IC/EC₅₀ values, derived from the pituitary cell culture assay, are the average of multiple experiments.

Binding to CRF-BP. The *K_i* values of CRF-BP for the various analogs presented in Table 1 were determined by the ability of the analogs to displace [D-¹²⁵I-Tyr⁴¹]hCRF as described (22). Inhibitory dissociation constants (*K_i*) were determined by using parameters calculated by the LIGAND computer program (23). Errors shown are the 95% confidence limits from three or more replicate binding assays.

Binding Assay. [D-¹²⁵I-Tyr⁴¹]astressin obtained by radioiodination of compound 4 was shown to bind with high affinity to CRF receptors (24). The *K_i* values listed in Table 1 were measured by using the cloned human CRF-RA1 (4, 24) stably expressed in CHO cells, as described (4). Errors are the 95% confidence limits from three or more binding assays.

Effect of the Astressin (Compound 3) on ACTH Secretion in Adrenalectomized Rats (see Fig. 2). Adult male rats were adrenalectomized under halothane anesthesia 8 days prior to the experiments. Their diet was supplemented with oranges, and their water contained 0.9% NaCl. They were equipped with indwelling jugular cannulae (25) 48 h prior to the i.v. injection of either vehicle or astressin. All protocols were approved by the Salk Institute Institutional Animal Care and Use Committee. Astressin was first diluted in sterile, distilled, pyrogenic water, and the pH was adjusted to 7.0. Further dilutions were made in 0.04 M phosphate buffer, pH 7.4, containing 0.1% bovine serum albumin and 0.01% ascorbic acid. Blood samples were obtained immediately before treatment, as well as 15–120 min later. Decanted plasma samples were frozen until assayed for ACTH concentrations with a commercially available kit (Allegro kit, Nichols Institute, San Juan Capistrano, CA) (25). Data were analyzed by analysis of variance followed by Duncan's multiple-range test for individual differences. Each bar represents the mean \pm SEM of six animals. *, *P* < 0.05; **, *P* < 0.01 from time 0.

Comparison Between the Ability of Compound 1 and Astressin to Interfere with Electroshock-Induced ACTH Secretion in Intact Male Rats (see Fig. 3). Intact male rats were equipped with indwelling jugular cannulae (25). On the day of the assay, rats were placed in individual boxes, and mild electroshocks (1 mA, 1-sec duration, 2 shocks per min) were delivered to their paws for 30 min. Control animals did not receive shocks. Other details are described above. Each bar represents the mean \pm SEM of seven animals.

RESULTS AND DISCUSSION

All analogs shown in Table 1 were synthesized by using the solid-phase approach and the Boc strategy with orthogonal protection (Fmoc and Ofm) of the side chains of residues to be cyclized (17). Best results were obtained when the peptide chain was assembled in its entirety prior to cleavage of the Fmoc and Ofm protecting groups and HBTU-mediated cyclization. The peptides were cleaved and deprotected in HF and purified by reversed-phase HPLC and an assortment of solvent systems (21). While the synthesis of the different CRF analogs described in Table 1 did not offer major difficulties, it should be noted that we have also isolated major components of our synthetic mixtures that presented with the desired masses minus 18. We strongly suspect the formation of an aspartimyl ring to be responsible for loss of one molecule of water (26). The analytical techniques used for the characterization of the analogs (Table 1) included HPLC with two different solvent systems (acidic and neutral) (21), amino acid analysis (data not shown), CZE, and LSIMS. Results from these studies support the identity of the intended structures. All peptides were ~95% pure.

Upon concluding that an *i*-(*i* + 3) Glu to Lys lactam bridge was compatible with α -helix formation and most likely was the best possible bridging moiety to fulfill increased structural stability, we completed the synthesis of an *i*-(*i* + 3) bridge scan Glu-Xaa-Xaa-Lys (A. Miranda, S.L.L., G.-C. Jiang, C.R., S.C.K., A.C., A.G.C., W.V., and J.R., unpublished data) of compound 1, which is one of the most potent CRF antagonists reported to date (12). Here, we show that the introduction of this bridge at positions 30 to 33 results in an antagonist with a solution conformation characterized by a strong positive absorbance below 195 nm, a distinct negative absorbance at 204 nm, and a characteristic negative shoulder at approximately 222 nm by CD spectroscopy in aqueous solution (Fig. 14). The intensity of these absorbances is characteristic of α -helices. Only subtle differences among the CD spectra of compounds 1, 3, and 5 could be detected. Comparison of the positive ellipticity at 190 nm suggests graded increase in α -helical content upon introduction of the Glu³⁰ and Lys³³ substitutions (compound 5 with potential salt bridge formation) and closure of the lactam ring found in compound 3. The TFE CD spectra of compounds 1, 3, and 5 suggest that all three compounds assume equivalent highly helical conformations (Fig. 18).

Astressin shows remarkably high potency in our pituitary cell culture assay, with high affinity for cloned human CRF-RA1 stably expressed in CHO cells and high potency to inhibit ACTH secretion when administered both *in vitro* and *in vivo*. Although we had anticipated that an *i*-(*i* + 3) Glu-Xaa-Xaa-Lys lactam ring would possibly stabilize a helix, we had not expected such dramatic improvement in potency in the antagonist series (compounds 3 and 4). We then investigated whether changes in potency resulted from the specific substitutions at position 30 and 33 (Glu and Lys, respectively) (compound 5) or the actual formation of the lactam constraint (compound 3). The effect of this modification or substitutions in agonists (compound 7) was also studied (compounds 8 and 9, respectively).

Astressin was found to be 32 times more potent than our present standard at inhibiting ACTH secretion *in vitro*, and 100 times more potent than α -helical CRF-(9-41). The corresponding linear analog (compound 5) is 1/300th as potent, suggesting that the Glu³⁰ to Lys³³ lactam bridge is essential in constraining the bioactive conformation. It should be noted that while compound 3 is more potent than compound 1, which itself is more potent than compound 5, CD data in aqueous buffer suggest that compound 3 is more helical than compound 5, which itself is more helical than compound 1. This apparent lack of correlation between relative potency/binding affinity

Table 1. Chemical and biological characterization of CRF analogs

No.	Structure	% purity by CZE	Mass, Da		Specific rotation	Relative potency, ACTH secretion	IC/EC ₅₀ , nM	K _i , nM	
			Found	Calculated				CRF-BP	hCRF-RA1
1	[D-Phe ¹² , Nle ^{21,23}]hCRF-(12-41)	99	3538.0	3538.01	-64.0	1.0	25.4 ± 3.9	310 (270-370)	56 (41-75)
2	α -helical CRF-(9-41)	91	3825.2	3824.97	+1.0	0.03 (0.02-0.05)	373.7 ± 36.5	0.067 (0.032-0.14)	17 (13-21)
3	cyclo(30-33)[D-Phe ¹² , Nle ^{21,23} , Glu ³⁰ , Lys ³³]hCRF-(12-41)*	97	3562.2	3562.05	-62.4	32.5 (12-82)†	1.0 ± 0.5	>1000	2.0 (1.8-2.3)
4	cyclo(30-33)[D-Tyr ¹¹ , Nle ^{21,23} , Glu ³⁰ , Lys ³³]hCRF-(12-41)	94	3578.2	3578.04	-59.0	5.6 (3.4-9.4)	7.0 ± 2.5	>1000	2.0 (1.4-2.9)
5	[D-Phe ¹² , Nle ^{21,23} , Glu ³⁰ , Lys ³³]hCRF-(12-41)	94	3580.1	3580.06	-59.1	0.10 (0.06-0.16)	264.2 ± 32.6	>1000	525 (400-630)
6	r/hCRF‡	96	4755.7	4755.51	-54.0	1.0	0.027 ± 0.003	0.12 (0.11-0.14)	6.9 (4.5-11)
7	[Ac-Pro ⁴ , D-Phe ¹² , Nle ^{21,23}]r/hCRF-(4-41)	95	4441.5	4441.48	-44.0	2.6 (1.4-4.8)	0.012 ± 0.004	5.1 (4.5-5.7)	1.6 (1.1-2.3)
8	cyclo(30-33)[Ac-Pro ⁴ , D-Phe ¹² , Nle ^{21,23} , Glu ³⁰ , Lys ³³]hCRF-(4-41)	95	4440.8	4440.52	-38.9	6.3 (3.2-12.9)	0.006 ± 0.001	4.4 (3.8-5.1)	1.1 (0.71-1.6)
9	[Ac-Pro ⁴ , D-Phe ¹² , Nle ^{21,23} , Glu ³⁰ , Lys ³³]hCRF-(4-41)	95	4458.6	4458.54	-39.5	4.5 (2.7-7.6)	0.008 ± 0.002	6.1 (5.4-7.0)	4.2 (3.0-5.8)

*Compound 3 is stressin.

†Average from four assays.

‡Sequence of r/hCRF:

1 Ser-Glu-Glu-Pro-Phe-Ile-Ser-Leu-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-Glu-Ala-Glu-Gln-Leu-Ala-Gln-Ala-His-Ser-Asn-Arg-Lys-Leu-Met-Glu-Ile-Ile-NH₂.

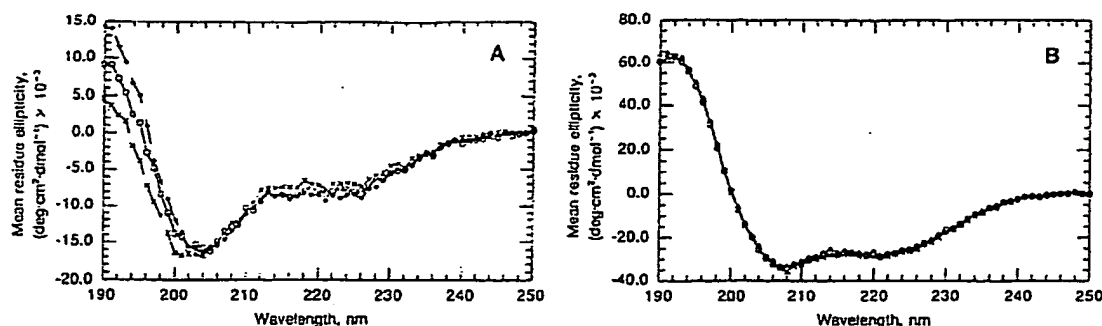


FIG. 1. CD spectra of compounds 1, 3, and 5 in aqueous solution (A) or in trifluoroethanol (B). Conditions are given in *Materials and Methods*. Compound 1, X; compound 3, ♦; compound 5, ○.

(Table 1) and structure determined by CD cannot be explained at this time, yet it is not without precedent (12, 16). While receptor/membrane mediated effects are poorly understood at this time, more rigorous structural data obtained by using NMR and possibly x-ray diffraction could suggest more refined and testable hypotheses for our understanding of CRF structure activity relationships.

Interestingly, astressin at the highest doses tested showed less intrinsic activity than either compound 1 or 2 and, therefore, was expected to be more effective *in vivo* than compound 1 when tested in two *in vivo* assays. Results of the effects of two low doses of astressin on ACTH secretion in adrenalectomized rats are shown in Fig. 2. The fact that 30 μ g and 100 μ g of astressin per kg administered i.v. still produced a significant decrease in ACTH levels at 45 and 90 min, respectively, was unexpected in view of the lack of a similar effect of 3 mg of compound 1 per kg. A dose of 300 μ g of astressin per kg was necessary for consistent and complete inhibition at the 90-min time point (data not shown). No significant inhibition was observed at the 120-min time point with any of the doses tested. We also tested the ability of astressin to inhibit stress-induced ACTH release in intact rats. Astressin was at least 10 times more potent than compound 1 in decreasing ACTH levels 10 and 30 min after initiation of the stress (Fig. 3). Whether these results can be explained primarily by the fact that astressin has greater affinity for the receptor or greater affinity/metabolic stability than the standard is not clear. Collectively, these data suggest that astressin is >10 times more potent after peripheral administration *in vivo* than any other antagonist reported to date and may be a reasonable candidate for exploratory clinical investigations.

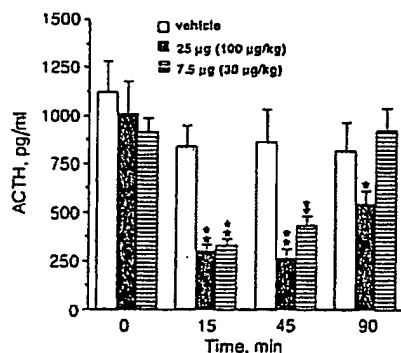


FIG. 2. Effect of astressin on ACTH secretion in adrenalectomized rats. Conditions are given in *Materials and Methods*.

An antagonist suitable for iodination is essential for future receptor studies; compound 4 was synthesized and found to be less potent than astressin in the rat anterior pituitary cell culture assay. The iodinated analog binds with high affinity, similar to that of astressin, in a membrane receptor assay (Table 1) and appears to be an ideal ligand for binding studies (24). From the data in Table 1, it is seen that the new cyclic antagonists have affinities for the cloned pituitary receptor that are equal to or greater than 10 times those of the first generations of CRF antagonists and three times that of r/hCRF.

The Glu³⁰ to Lys³³ lactam bridge was also introduced in compound 7, one of the most potent CRF agonists. The limited 2-fold increase in potency for both the cyclic (compound 8) and the linear (compound 9) analogs raises the possibility that the N terminus of CRF fulfills two independent roles: to activate the receptor and to induce strong α -helicity along the whole CRF molecule. Indeed, the fact that both linear and cyclic peptides are statistically equipotent suggests that the bridge does not contribute significantly to the stabilization of the overall bioactive conformation of the agonist.

hCRF loses its ability to stimulate hypophyseal ACTH release when binding to the CRF-BP (affinity of hCRF for CRF-BP is \sim 10 times greater than that for the CRF receptor) (22). Therefore, we anticipate that any antagonist with high binding affinity to the CRF-BP may be less efficacious *in vivo* when CRF-BP is present. We found in a systematic structure activity relationship study that CRF-BP binds hCRF and carp urotensin I with high affinity, sauvagine with moderate affi-

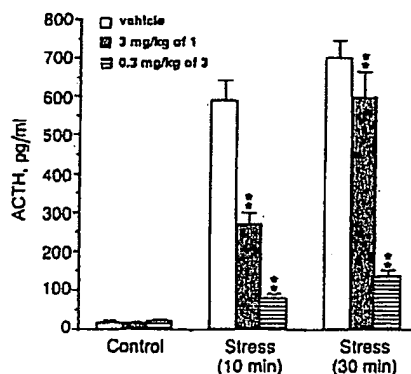


FIG. 3. Comparison between the ability of compound 1 and astressin (compound 3) to interfere with electroshock-induced ACTH secretion in intact male rats. Conditions are given in *Materials and Methods*.

ity, and oCRF with low affinity (22). Among the antagonists reported to date, compound 2 has high affinity for CRF-BP while compound 1 has low affinity. Since residues Asp⁹-Leu¹⁰-Thr¹¹ seemed to be important for binding, it was expected that compounds 3-5 would have low affinity for the binding protein, as shown in Table 1. Accordingly, the agonists (compounds 7-9) have 1/40th the affinity for the CRF-BP as compared with r/hCRF, with the influence of the cycle being insignificant (compare the affinity of compound 7 for CRF-BP with that of 8).

Prior generations of CRF antagonists have been administered at high concentrations in the central nervous system and shown to effectively blunt endogenous CRF actions; however, antagonists that would be potent and long acting on ACTH secretion have been lacking. Astressin is a significant improvement over previously available CRF antagonists due to its high potency, low intrinsic activity, high receptor affinity, and high solubility in neutral aqueous solutions.

Because astressin is effective at low doses, the impact of its limited intrinsic activity at high concentrations *in vitro* may be of limited consequence *in vivo*. Also, the availability of a high affinity antagonist radioligand will now allow further advances in receptor pharmacology. These results illustrate the role secondary and tertiary structures may play in controlling biological signaling through protein-ligand interactions. To our knowledge, there is no documented evidence for such long distance induction/stabilization (imparted by residues 4-11) of α -helix formation that can be restored by a single bridging element 20 residues down the sequence upon deletion of these residues. We have shown that a Glu/Lys $i-(i+3)$ and $i-(i+4)$ lactam bridge may impart the proper geometry for the stabilization of an α -helical backbone (16). Additionally, Creamer and Rose (27) have concluded on the basis of changes in configurational entropy of a residue side chain upon transfer from an unfolded state to the an α -helical state that "general factors that drive helix formation must originate in the backbone" (27). We conclude, therefore, that it is likely that the increased potency of astressin compared with that of compound 5 is due to the added, lactam ring-induced constraints of the backbone that lead to a stabilized α -helix extending the whole length of the fragment in the receptor environment. Finally, because the CRF receptors belong to an extended subfamily of receptors for peptides, including vasoactive intestinal peptide (28), calcitonin (29), secretin (30), parathyroid hormone (31), and growth hormone-releasing factor (32), the possibility remains that these hormones with truncated N termini may be stabilized in a similar manner to that of astressin for the generation of potent antagonists.

We thank Drs. G. Koob and C. Hoeger for constructive comments. We thank C. Miller, R. Kaiser, D. Pantoja, Y. Haas, and S. Leonard for technical assistance and D. Johns for manuscript preparation. This work was supported in part by National Institutes of Health Grant DK-26741, the Hearst Foundation, and the Foundation for Research, California Division. W.V., C.R., and A.G.C. are Foundation for Research investigators.

- Vale, W., Spiess, J., Rivier, C. & Rivier, J. (1981) *Science* 213, 1394-1397.
- Vale, W., Vaughan, J., Yamamoto, G., Bruhn, T., Douglas, C., Dalton, D., Rivier, C. & Rivier, J. (1983) In *Assay of Corticotropin Releasing Factor*, ed. Conn, P. M. (Academic, New York), Vol. 103, pp. 565-577.
- Taché, Y. & Rivier, C., eds. (1993) In *Corticotropin-Releasing Factor and Cytokines: Role in the Stress Response*, Proceedings of the Hans Selye Symposium on Neuroendocrinology and Stress (NY Acad. Sci., New York), Vol. 697.
- Chen, R., Lewis, K. A., Perrin, M. H. & Vale, W. W. (1993) *Proc. Natl. Acad. Sci. USA* 90, 8967-8971.
- Chang, C. P., Pearce, R. V., II, O'Connell, S. & Rosenfeld, M. G. (1993) *Neuron* 11, 1187-1195.
- Perrin, M., Donaldson, C., Chen, R., Blount, A., Berggren, T., Bilezikjian, L., Sawchenko, P. & Vale, W. (1995) *Proc. Natl. Acad. Sci. USA* 92, 2969-2973.
- Kishimoto, T., Pearce, R. V., II, Lin, C. R. & Rosenfeld, M. G. (1995) *Proc. Natl. Acad. Sci. USA* 92, 1108-1112.
- Vita, N., Laurent, P., Lefort, S., Chalou, P., Lelias, J. M., Kaghnd, M., Le, F. G., Caput, D. & Ferrara, P. (1993) *FEBS Lett.* 335, 1-5.
- Lovenberg, T. W., Liaw, C. W., Grigoriadis, D. E., Clevenger, W., Chalmers, D. T., DeSouza, E. B. & Oltersdorf, T. (1995) *Proc. Natl. Acad. Sci. USA* 92, 836-840.
- Perrin, M. H., Donaldson, C. J., Chen, R., Lewis, K. A. & Vale, W. W. (1993) *Endocrinology* 133, 3058-3061.
- Potter, E., Behan, D. P., Fischer, W. H., Linton, E. A., Lowry, P. J. & Vale, W. W. (1991) *Nature (London)* 349, 423-426.
- Hernandez, J.-F., Kornreich, W., Rivier, C., Miranda, A., Yamamoto, G., Andrews, J., Taché, Y., Vale, W. & Rivier, J. (1993) *J. Med. Chem.* 36, 2860-2867.
- Rivier, J., Rivier, C. & Vale, W. (1984) *Science* 224, 889-891.
- Romier, C., Bernassau, J.-M., Cambillau, C. & Darbon, H. (1993) *Protein Eng.* 6, 149-156.
- Pallai, P. V., Mabilia, M., Goodman, M., Vale, W. & Rivier, J. (1983) *Proc. Natl. Acad. Sci. USA* 80, 6770-6774.
- Miranda, A., Koerber, S. C., Gulyas, J., Lahrichi, S., Craig, A. G., Corrigan, A., Hagler, A., Rivier, C., Vale, W. & Rivier, J. (1994) *J. Med. Chem.* 37, 1450-1459.
- Felix, A. M., Heimer, E. P., Wang, C. T., Lambrus, T. J., Fournier, A., Mowles, T. F., Maines, S., Campbell, R. M., Wegrzynski, B. B., Toome, V., Fry, D. & Madison, V. S. (1988) *Int. J. Pept. Protein Res.* 32, 441-454.
- Cervini, L. A., Corrigan, A., Donaldson, C. J., Koerber, S. C., Vale, W. W. & Rivier, J. E. (1994) In *Cyclic Analogs of [MeTyr¹, Ala¹⁵, MeTyr²⁷]GRF(1-29)-NH₂ with High Potencies in Vitro*, eds. Hodges, R. S. & Smith, J. A. Vol. (ESCOM, Leiden, The Netherlands), pp. 541-543.
- Felix, A. M., Wang, C. T., Heimer, E. P. & Fournier, A. (1988) *Int. J. Pept. Protein Res.* 31, 231-238.
- Rivier, J. (1978) *J. Liq. Chromatogr.* 1, 343-367.
- Hoeger, C., Galyean, R., Boublik, J., McClintock, R. & Rivier, J. (1987) *Biochromatography* 2, 134-142.
- Sutton, S. W., Behan, D. P., Lahrichi, S., Kaiser, R., Corrigan, A., Lowry, P., Potter, E., Perrin, M., Rivier, J. & Vale, W. W. (1995) *Endocrinology* 136, 1097-1102.
- Munson, P. J. & Rodbard, D. (1980) *Anal. Biochem.* 107, 220-239.
- Perrin, M. H., Sutton, S., Gulyas, J., Lovejoy, D., Rivier, J. E. & Vale, W. W. (1995) *Soc. Neurosci. Abstr.* 21, 1390.
- Rivier, C. & Shen, G. H. (1994) *J. Neurosci.* 14, 1985-1993.
- Tam, J. P., Riemen, M. W. & Merrifield, R. B. (1988) *Peptide Res.* 1, 6-18.
- Creamer, T. P. & Rose, G. D. (1992) *Proc. Natl. Acad. Sci. USA* 89, 5937-5941.
- Ishihara, T., Shigemoto, R., Mori, K., Takahashi, K. & Nagata, S. (1992) *Neuron* 8, 811-819.
- Lin, H. Y., Harris, T. L., Flannery, M. S., Aruffo, A., Kaji, E. H., Gorn, A., Kolakowski, L. F., Jr., Lodish, H. F. & Goldring, S. R. (1991) *Science* 254, 1022-1024.
- Ishihara, T., Nakamura, S., Kaziro, Y., Takahashi, K. & Nagata, S. (1991) *EMBO J.* 10, 1635-1641.
- Segre, G. V. & Goldring, S. R. (1993) *Trends Endocrinol. Metab.* 4, 309-314.
- Gaylinn, B. D., Harrison, J. K., Zysk, J. R., Lyons, C. E., Lynch, K. R. & Thorne, M. O. (1993) *Mol. Endocrinol.* 7, 77-84.

Recent Advances with the CRF₁ Receptor: Design of Small Molecule Inhibitors, Receptor Subtypes and Clinical Indications

James R. McCarthy*, Stephen C. Heinrichs and Dimitri E. Grigoriadis

Neurocrine Biosciences, Inc., San Diego, CA 92121, USA

Abstract: Corticotropin-releasing factor (CRF) has been widely implicated as playing a major role in modulating the endocrine, autonomic, behavioral and immune responses to stress. The recent cloning of multiple receptors for CRF as well as the discovery of non-peptide receptor antagonists for CRF receptors have begun a new era of CRF study. Presently, there are five distinct targets for CRF with unique cDNA sequences, pharmacology and localization. These fall into three distinct classes, encoded by three different genes and have been termed the CRF₁ and CRF₂ receptors (belonging to the superfamily of G-protein coupled receptors) and the CRF-binding protein. The CRF₂ receptor exists as three splice variants of the same gene and have been designated CRF_{2α}, CRF_{2β} and CRF_{2γ}. The pharmacology and localization of all of these proteins in brain has been well established. The CRF₁ receptor subtype is localized primarily to cortical and cerebellar regions while the CRF_{2α} receptor is localized to subcortical regions including the lateral septum, and paraventricular and ventromedial nuclei of the hypothalamus. The CRF_{2β} receptor is primarily localized to heart, skeletal muscle and in the brain, to cerebral arterioles and choroid plexus. The CRF_{2γ} receptor has most recently been identified in human amygdala. Expression of these receptors in mammalian cell lines has made possible the identification of non-peptide, high affinity, selective receptor antagonists. While the natural mammalian ligands oCRF and r/hCRF have high affinity for the CRF₁ receptor subtype, they have lower affinity for the CRF₂ receptor family making them ineffective labels for CRF₂ receptors. [¹²⁵I]Savagine has been characterized as a high affinity ligand for both the CRF₁ and the CRF₂ receptor subtypes and has been used in both radioligand binding and receptor autoradiographic studies as a tool to aid in the discovery of selective small molecule receptor antagonists. A number of non-peptide CRF₁ receptor antagonists that can specifically and selectively block the CRF₁ receptor subtype have recently been identified. Compounds such as CP 154,526 (12), NBI 27914 (129) and Antalarmin (154) inhibit CRF-stimulation of cAMP or CRF-stimulated ACTH release from cultured rat anterior pituitary cells. Furthermore, when administered peripherally, these compounds compete for *ex vivo* [¹²⁵I]savagine binding to CRF₁ receptors in brain sections demonstrating their ability to cross the blood-brain-barrier. In *in vivo* studies, peripheral administration of these compounds attenuate stress-induced elevations in plasma ACTH levels in rats demonstrating that CRF₁ receptors can be blocked in the periphery. Furthermore, peripherally administered CRF₁ receptor antagonists have also been demonstrated to inhibit CRF-induced seizure activity. These data clearly demonstrate that non-peptide CRF₁ receptor antagonists, when administered systemically, can specifically block central CRF₁ receptors and provide tools that can be used to determine the role of CRF₁ receptors in various neuropsychiatric and neurodegenerative disorders. In addition, these molecules will prove useful in the discovery and development of potential orally active therapeutics for these disorders.

Introduction

Hypothalamic extracts were first demonstrated in the mid 1950's by two independent groups to contain a specific factor, termed corticotropin-releasing factor (CRF), that was capable of stimulating pituitary secretion of adrenocorticotrophic hormone (ACTH) [1, 2]. Although CRF was the first hypothalamic hypophysiotropic factor to be recognized functionally, its chemical identity remained unsolved for several years. The development of specific radioimmunoassays for ACTH and quantitative *in vitro* methods for assaying hypophysiotropic substances, along with the utilization of ion-exchange and HPLC techniques led to the successful purification isolation, characterization and synthesis of a functional 41-amino acid CRF from sheep hypothalamic extracts in 1981 by Vale and co-workers at the Salk Institute [3].

The sequence of CRF has been determined in a variety of species including sheep, man, rats, pigs, goats and cows. In all species, CRF is a 41-amino acid residue single chain polypeptide. Rat and human CRF are identical to one another and differ from ovine CRF by seven amino acid residues. All three CRFs have close amino acid homology and share some biological properties with sauvagine, a 40-amino acid peptide that exists in frog skin, and urotensin I, a 41-amino acid peptide derived from fish urophysis. A novel mammalian form of urotensin I termed "urocortin", that shares many of the *in vitro* and *in vivo* functions of CRF, has been identified and the gene sequenced and characterized [4]. Interestingly, although differences exist in the amino acid sequences of the various CRF-like peptides, all these peptides share one common feature. The carboxy terminus of these peptides must be amidated for biological activity. Indeed, deamidation of CRF to the CRF-COOH terminal free acid retains less than 0.1% of the potency of native CRF suggesting the importance of this functional group. Using proton nuclear magnetic resonance, the solution structure of CRF has been elucidated and suggests that CRF is comprised of an extended N-terminal tetrapeptide connected to a

*Address correspondence to this author at Neurocrine Biosciences, Inc., 10555 Science Center, San Diego, CA 92121, USA; Tel.: (619) 658-7630; Fax: (619) 658-7601; Email: jmccarthy@neurocrine.com

well-defined α -helix between residues 6 to 36 [5]. Truncated forms of CRF such as α -helical ovine CRF(9-41) have been demonstrated to be antagonists at CRF receptors [6] and further underscores the necessity of the α -helical conformation for receptor binding and biological activity.

Utilizing a variety of techniques including Northern blot analysis, in situ hybridization histochemistry, radioimmunoassay and immunohistochemistry, CRF has been shown to have a widespread, unique and specific distribution within the central nervous system. Clearly the major site of CRF localization is the hypothalamus where it mediates its hypophysiotropic effects on the pituitary gland, but the identification of extrahypothalamic CRF has defined a role for this peptide as a *bona fide* neurotransmitter. For example, the neocortex contains primarily CRF interneurons with bipolar, vertically oriented cell bodies predominantly localized to the second and third layers of the cortex and fiber projections to layers I and IV. In addition, scattered cells are present in the deeper layers which appear to be pyramidal cells. The CRF neurons in the cerebral cortex appear to be important in several behavioral actions of the peptide including effects on cognitive processing. Furthermore, dysfunction of these neurons may contribute to many CNS disorders. Other major sites of large and discrete populations of CRF perikarya include the central nucleus of the amygdala, the bed nucleus of the stria terminalis and the substantia innominata. The CRF neurons in the central nucleus of the amygdala project to the parvocellular regions of the PVN, the parabrachial nucleus of the brain stem and therefore may influence both neuroendocrine and autonomic function in addition to behavioral activity.

Within the spinal cord, CRF cell bodies are present in laminae V to VII and X and in the intermediolateral column of the thoracic and lumbar cord. CRF fibers originating in the spinal cord form an ascending system terminating in the reticular formation, the vestibular complex, the central gray and the thalamus. This ascending CRF system may play an important role in modulating sensory input. In addition, spinal cord CRF neurons may represent preganglionic neurons that modulate sympathetic outflow. Detailed reviews are available describing the discrete distribution and localization of CRF-immunoreactive cells and fibers [7-9].

In addition to the central distribution of CRF, a variety of peripheral tissues also contain CRF [10]. For example CRF-like immunoreactive fibers are present in the intermediate lobe of the pituitary gland where they are proposed to regulate proopiomelanocortin (POMC)-derived peptide secretion from that tissue. CRF has also been localized in the adrenal medulla and has been reported to be increased following stimulation of the splanchnic nerve and hemorrhagic stress. CRF-like immunoreactivity and CRF mRNA have been detected in lymphocytes where they may play a role in regulating immune function. In addition, very recently, effects of CRF and urocortin have been observed in mast cell degranulation leading to the hypothesis that CRF receptor may also play a major role in stress-induced immune disorders such as atopic dermatitis or psoriasis [11, 12]. Other tissues in which CRF has been localized include the testis (Leydig cells and advanced germ cells), pancreas, stomach and small intestine. While CRF is not detected in the circulation under normal circumstances, very high levels have been measured in the plasma of pregnant women; the source of CRF in pregnancy appears to be the placenta [13-15].

CRF Receptors

The study of CRF receptors for the past decade has been limited by the availability of selective ligands for the receptors, primarily iodine-125-labeled analogs of either rat/human or ovine CRF. The characteristics of this high affinity binding have been described in numerous publications [for reviews see 16, 17-19]. Equilibrium binding using [125 I]CRF in a variety of tissues has demonstrated the presence of a single high affinity binding site with an apparent dissociation constant (K_D) of 200-400 pM. The relative density of CRF receptors in various tissues is highest in the anterior and intermediate lobes of the pituitary, with moderate densities present in discrete areas of brain such as cerebral cortex and cerebellum, and low, but detectable, densities of receptors present in spleen. Although the identification and characterization of the CRF receptors fulfilled all the criteria for a complete neurotransmitter-hormone system, the receptor proteins identified and localized could not fully account for all the actions of the transmitter in either the central nervous system or in the periphery.

Several groups simultaneously reported the cloning of cDNAs from rat, mouse, and human which encoded a CRF receptor (CRF₁) [20-23]. The mRNA distribution for the CRF₁ receptor correlated well with the known distribution of CRF binding sites in that expression was highest in the pituitary, cerebral cortex, and cerebellum. Indeed, when this receptor was expressed in cells, it exhibited an identical pharmacological profile to that previously described in brain and pituitary. Furthermore, we and others have reported the cloning of a second member of the CRF receptor family (CRF₂) from both rat [24] and mouse [25, 26]. Initially, only two apparent splice forms of this receptor subtype were identified, termed CRF_{2 α} and CRF_{2 β} . Both of these forms were reported in rat [24], however, only the CRF_{2 β} form was described in mouse [25, 26]. Subsequently, the cDNA for the human form of the CRF_{2 γ} receptor was cloned from human amygdala [27]. The anatomical distribution of the CRF₂ receptor in the rat is completely distinct from that of the CRF₁ receptor [28]. In addition, CRF₂ receptors display a unique pharmacological profile when expressed in cells which further serves to separate and distinguish them from the CRF₁ receptor subtype [29]. These differences in the anatomical and pharmacological profile, coupled with the lack of molecules available as tools with high affinity for the CRF₂ receptor subtypes, are primarily responsible for the inability to clearly elucidate the physiologic nature of this family of receptors. As mentioned above, until now, the assessment of these receptor subtypes was limited by the availability of suitable radioligand probes. With the characterization and development of [125 I]Savagine as a radioligand which has high affinity for both subtypes of CRF receptors [30] coupled with the specific CRF₁ non-peptide receptor antagonists described below, the selective study of the CRF₂ receptor can now be performed.

The availability of nucleotide sequences for CRF₁ and CRF₂ receptors has allowed a detailed examination of the regional and cellular distribution of CRF receptor subtype mRNA expression utilizing both RNase protection assays and in situ hybridization histochemistry. The results of these studies indicate heterogeneous distribution patterns of CRF receptor subtypes in brain and peripheral tissues suggestive of specific physiological roles for these sites in CRF-related functions. A complete and detailed account of the mRNA distribution patterns of the CRF₂ receptor has been reported [31]. This receptor subtype is currently being implicated in a variety of physiological processes ranging from higher cognitive functions such as learning and memory to

autonomic regulation, including food and water intake [32]. In addition, with the high expression of this subtype in areas such as the lateral septum and hypothalamic areas, the CRF₂ receptor may play a key function in mediating a variety of emotional conditions including fear and aggression. [33]. The lack of CRF₁ receptor expression in these nuclei suggests that CRF₂ receptors may solely mediate the postsynaptic actions of CRF inputs to this region and furthermore is evidence for a role for CRF₂ receptors in modulating limbic circuitry at the level of septal activity.

Pharmacological Characteristics

To date, there have been numerous publications that have described the radioligand binding characteristics of CRF receptors using the available radioligands in a variety of tissues [for review see 17, 34-36]. However, prior to the recent elucidation of the CRF₂ subfamily of receptors, all of the *in vitro* and *in vivo* characteristics were ascribed to a single receptor subtype. Fortunately, this body of data is unaffected by the discovery of a second family member by virtue of the fact that [¹²⁵I]r/hCRF and [¹²⁵I]oCRF have lower affinity for this subtype (10 - 100 nM) making them essentially selective tools for the CRF₁ receptor subtype. Thus, the commercially available radioligands for CRF receptors had relatively low affinity for the CRF₂ receptor subtype and were not useful in identifying and characterizing this subtype. As mentioned above, we synthesized and radioiodinated to a high specific activity, the peptide [¹²⁵I]Tyr⁰-sauvagine and used it to characterize the CRF₂ receptor subtype [30].

Using [¹²⁵I]Tyr⁰-sauvagine, the two receptor subtypes could be distinguished by their pharmacological rank order binding profile using a number of CRF-related peptides. This rank order binding profile was consistent with the *in vitro* effects of the same unlabeled peptides in the production of cAMP in cells expressing either subtype of the receptor. Thus, the non-mammalian analogs sauvagine and urotensin I that were more potent in stimulation of cAMP production from cells expressing the CRF₂ receptor, were also more potent at inhibiting the binding of [¹²⁵I]sauvagine than oCRF or r/hCRF. These data clearly suggest that although there is a distinct pharmacological difference between the two receptor subtypes of the same family (in terms of their rank order profile), they still must share some structural similarities. Further study will be required to determine the precise common structural features of these two family members. In addition to the non-mammalian CRF-like peptides having equal affinity for CRF₁ and CRF₂ receptors, the putative antagonists for CRF receptors, D-PheCRF(12-41) and α -helical CRF(9-41) also exhibited approximately equal affinity for the two receptor subtypes [30]. This further serves to not only identify these two subtypes as close family members but offers the opportunity for the design of molecules that can interact specifically with one or the other receptor or both.

Beyond the similarities between the two receptor proteins and the peptide affinities in the binding and cAMP profiles described above, the human CRF₂ receptor also conforms to the superfamily of G-protein coupled receptors. G-proteins are known to play an integral role in the regulation of receptor-mediated events for many receptor systems [for review see 37]. Consistent with CRF receptors being coupled to a guanine nucleotide regulatory protein, increasing the concentrations of GTP or its non-hydrolyzable analogs Gpp(NH)p and GTP- γ -S to the incubation medium resulted in an inhibition of [¹²⁵I]sauvagine binding in cells transfected with either the human CRF₁ or CRF_{2 α} receptor. This effect appeared to

be specific to the guanine nucleotides, as similar experiments indicated that equimolar concentrations of adenosine-5'-triphosphate (ATP) had very little effect on sauvagine binding.

Recently, a novel peptide ligand was identified and characterized as having high affinity for the CRF₂ receptor. This mammalian form of urotensin termed "urocortin" was localized by urotensin I-like immunoreactivity and subsequently cloned from rat brain [4]. This peptide exhibits 63% identity with the fish urotensin I and 45% identity with rat CRF. Although this peptide has high affinity for the CRF₁ receptor and produces the same effects *in vitro* and *in vivo* as does CRF itself [4, 38], it maintains its high affinity interaction for CRF₂ receptors similar to sauvagine and urotensin I. In addition, this molecule has high affinity for the CRF binding protein establishing a key role in the regulation of the CRF system. Thus, this molecule likely represents one endogenous ligand for the mammalian CRF_{2 α} receptor. Future studies will be aimed toward the identification and characterization of these and other ligands and receptors and will subsequently allow us to gain a greater awareness of the CRF system.

Neuropsychiatric Disorders Accompanied by CRF Overabundance

A number of clinical investigators have described a significant positive correlation between measures of brain CRF activation and the incidence and severity of affective and anxiety disorders. In particular, post-mortem CRF receptor density, endocrine reactivity of the HPA-axis to pharmacological challenge with CRF/corticosteroid and also cerebrospinal fluid detection of CRF all serve as measures of CRF system tone and dynamics. For example, the affective disorder, melancholic depression, is characterized by diminished density of brain CRF receptors, diminished sensitivity of the HPA-axis to CRF challenge and elevated cerebrospinal fluid levels of CRF all of which point to overactivation of brain CRF systems. Anxiety disorders such as post-traumatic stress disorder are also characterized by apparent overabundance of brain CRF as revealed by abnormally high levels of CRF in cerebrospinal fluid [39] although HPA-axis reactivity, in contrast to melancholic depression, appears to be diminished in post-traumatic stress disorder [40]. The degree to which affective and anxiety disorders, together with substance abuse disorder constitute a class of neuropsychiatric disorders with a common element of CRF system overactivation is considered below.

Depression and Anxiety

A number of observations suggest that CRF systems function abnormally in depressed patients. Many patients with major depression are hypercortisolemic and exhibit an abnormal dexamethasone suppression test [41]. Given the primary role of CRF in stimulating pituitary-adrenocortical secretion, it is possible that hypersecretion of CRF in brain might underlie the hypercortisolemia and symptomatology seen in major depression. The cerebrospinal fluid concentration of CRF is significantly elevated over normals in depressed patients [42], and a significant positive correlation is observed between CRF concentrations in the cerebrospinal fluid and the degree of insensitivity to dexamethasone suppression of plasma cortisol in depressed individuals [43]. Furthermore, the observation of a decrease in CRF binding sites in the frontal cerebral cortex of suicide victims compared to controls [42] is consistent with the hypothesis that CRF is hypersecreted in

major depression. The increased cerebrospinal fluid concentrations of CRF seen in depressed individuals are normalized by electroconvulsive therapy and correlate well with improvement [44].

In view of the data suggesting a role for CRF in depression, the hypothesis has been put forth that antidepressants may produce their therapeutic effects, in part, by decreasing CRF secretion. An increase in CRF binding sites, presumably to compensate for chronic suppression of CRF secretion, is observed in some brain regions such as the brain stem in rats treated chronically with tricyclic antidepressants such as imipramine [45]. Moreover, CRF is postulated to have an important role in the locus ceruleus of the brain stem. The locus ceruleus receives a rich CRF innervation [8], contains a moderate density of CRF receptors [17], and is markedly activated following central administration of CRF [46]. Furthermore, concentrations of CRF are selectively increased in the locus ceruleus following application of acute or chronic stress [47]. Given the major involvement of the brain noradrenergic system, in particular in the locus ceruleus in depression and the effects of CRF to activate noradrenergic neurons in this brain region [48], it is possible that antidepressants may function by suppressing CRF secretion in the locus ceruleus, resulting in the observed increase in brain stem CRF binding sites.

The role that has been proposed for CRF in major depressive disorders along with preclinical data in rats demonstrating effects of CRF administration to produce several behavioral changes characteristic of anxiogenic compounds [49] have led to the suggestion that CRF may also be involved in anxiety-related disorders. A role for CRF in panic disorder has been suggested by observations of blunted ACTH responses to intravenously administered CRF relative to control subjects [50]. The blunted ACTH response to CRF in panic disorder patients most likely reflects a process occurring at or above the hypothalamus, resulting in excess secretion of endogenous CRF. Similarly, anxiety disorders such as post-traumatic stress disorder are characterized by overabundance of brain CRF as revealed by abnormally high levels of CRF in cerebrospinal fluid [39]. The ability of CRF peptide infusion in animal models of emotionality to produce anxiogenic-like behaviors, fear and neuronal hyperexcitability [51, 52] provide valid evidence of a role for CRF overabundance in human clinical anxiety. Moreover, sleeping, eating and mating cycle disruption characteristic of human anxiety [53] is an expected consequence of CRF overabundance based on the behavioral pharmacology of CRF peptide administration in animal models.

Further support for the hypothesis linking the brain stress-axis with anxiety disorders comes from neurochemical, endocrine and receptor binding data documenting interactions between CRF and benzodiazepine anxiolytics. Acute administration of the triazolobenzodiazepines, alprazolam and adinazolam, results in increased hypothalamic concentrations of CRF while decreasing the concentrations of CRF in other brain regions, including locus ceruleus, amygdala, pyriform cortex and cingulate cortex [54]. Of particular interest is the finding that the two triazolobenzodiazepines exert effects on CRF concentrations in the locus ceruleus and hypothalamus that are opposite to CRF changes seen after stress. Chronic administration of diazepam, alprazolam, or adinazolam in rats results in significant decreases in CRF receptors in the frontal cerebral cortex and hippocampus and there is a trend for CRF receptors to be decreased in other brain areas and increased in the anterior pituitary [45]. The latter data demonstrating increases in CRF receptor concentrations support the

hypothesis for effects of the benzodiazepines to inhibit CRF release which in turn modulate the receptors. Further support for this hypothesis is provided by potent *in vitro* effects of benzodiazepines [55] to inhibit hypothalamic CRF release. Conversely, the reduced concentrations of CRF in the other brain regions described above following *in vivo* administration of the benzodiazepines may relate to increased release of CRF, which would be expected to decrease receptors in regions like the frontal cortex and hippocampus. In view of the evidence described above suggesting that hypersecretion of CRF may underlie some of the symptomatology seen in affective disorders and anxiety-related disorders, it stands to reason that CRF receptor antagonists may be useful in the treatment of these disorders. Thus, a CRF antagonist may be a useful antidepressant, anxiolytic or anti-stress drug.

Substance Abuse

Stressful aspects of drug exposure in the drug-naïve organism may produce activation of brain CRF systems [56]. Cocaine is a robust activator of the HPA axis [57]. Increased cardiovascular effects and craving in response to cocaine self-administration in cocaine abusers has also been observed [58]. Cocaine also causes a rapid and robust increase in ACTH in cocaine dependent males [59]. Although stress responses in cocaine abusers have not been examined, a recent study with cocaine dependent individuals showed significant increases in plasma ACTH and cortisol with cocaine cue exposure [60]. Finally, increased levels of corticosterone are associated with increases in self-administration of cocaine and amphetamines [61]. These data suggest that acute stress, like cocaine, activates the HPA axis which has been shown to increase cocaine seeking and cocaine self-administration. These findings are the basis for the hypothesis that stress will increase physiological measures as well as neuroendocrine activity, as assessed by beta-endorphin, ACTH and cortisol levels, and increase craving in cocaine abusers.

In support of CRF receptor selective mediation of cocaine seeking behavior, a very recent report describes attenuation of stress-induced reinstatement of cocaine self-administration following pre-treatment with a CRF₁ receptor antagonist [62]. Moreover, significant, regionally selective and dose-dependent increases in hypothalamic CRF peptide content and CRF mRNA expression are observed following acute or "binge"-pattern administration of cocaine in rats [63]. This cocaine-induced CRF activation state appears to be critical for mobilizing adaptive responses, such as adrenocorticotropin secretion, to stressor exposure [56, 57]. A generalized involvement of CRF systems in drug-related, and in particular drug withdrawal-related, negative motivational states is consistent with the comprehensive role of CRF in mediating the coordinated response to environmental stressors [49]. Since the stressful behavioral and physiological aspects of cocaine persist in the cocaine-experienced organism and since anxiogenic-like responses are readily apparent following cocaine withdrawal [64], it seems reasonable to hypothesize that biological substrates responsible for behavioral and physiological responses to stress may play a role in cocaine seeking behavior.

Several lines of evidence from preclinical and clinical studies support the importance of stress and negative mood in perpetuating drug use and relapse. In laboratory animals, exposure to stressors has been shown to increase self-administration of amphetamines [61], morphine [65] and cocaine [66]. Brief footshock stress also results in reinstatement of drug-seeking behavior in heroin-

experienced, drug-free animals and in cocaine-experienced, drug-free animals [65]. In clinical surveys, drug abusers and alcoholics frequently cite psychological distress and negative mood states as reasons for relapse to drug use [67]. Although the relationship between stress and cocaine use has only recently been investigated, there are several lines of evidence suggesting a positive relationship between stress and cocaine use. First, diagnostic studies have shown a strong association between mood disorders and cocaine abuse [68]. Such findings have led to the well-known hypothesis that drug abusers may often use drugs to blunt or self-medicate negative mood states. Second, data relating negative affect experiences and stressful life events in alcoholics appear to be significantly related to relapse. For example, Cooney and colleagues [69] have shown that negative affect-induced alcohol cue reactivity predicts time to relapse after inpatient alcohol treatment in abstinent alcoholics. Brown et al. [70] also found that male alcoholics who relapsed had significantly more alcohol independent highly threatening events and severe ongoing difficulties than those who remained abstinent post-treatment. Finally, a positive relationship between internal negative mood states and drug craving has been demonstrated in laboratory studies with alcoholics, opiate abusers and smokers. Childress et al. [71] conducted an early study examining the effects of negative mood states and external heroin cues in opiate addicts. Their findings indicated that negative mood induction increased craving for opiates, and external drug cues provided no additional increases in craving. Litt et al. [72] were among the first to demonstrate that hypnotically-induced relevant negative mood states without the presence of cues elicited desire for alcohol among alcoholics. Using guided imagery to induce positive or negative affect, Tiffany & Drobes [73] also demonstrated mood-induced craving for cigarettes in smokers. Additional studies with alcoholics and smokers have replicated these findings [69].

Overproduction of CRF in Transgenic Mice: A Genetic Model of Anxiogenic Behavior

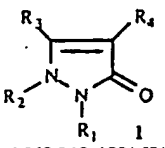
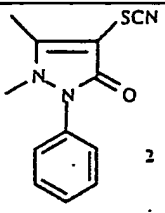
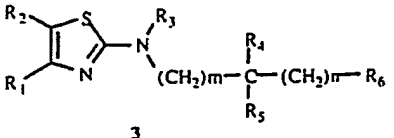
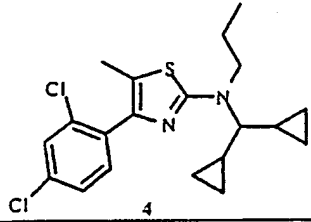
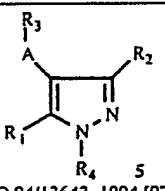
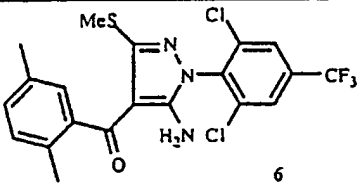
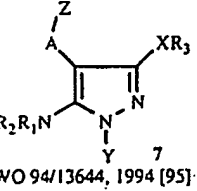
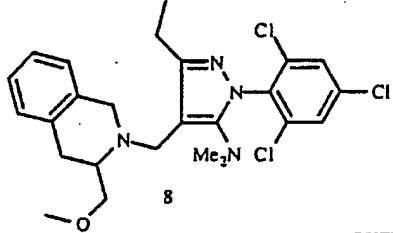
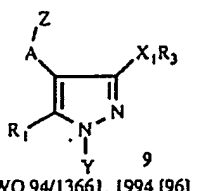
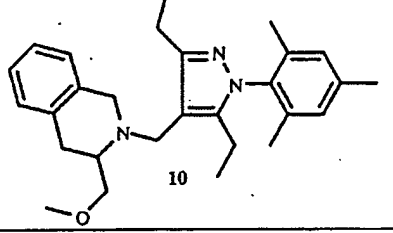
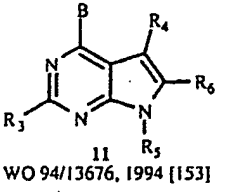
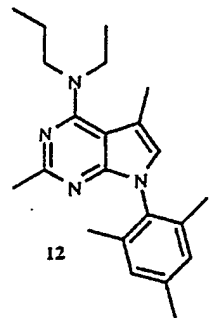
CRF overproduction has been hypothesized to be involved in a number of stress-related psychiatric disorders including anxiety and affective disorders. A transgenic mouse model of CRF overproduction has been developed [74] using a CRF transgene composed of rat genomic CRF. The CRF transgenic mice not only exhibit endocrine abnormalities including elevations of ACTH and corticosterone, but also enhanced reactivity to novelty and an anxiogenic-like response in animal models of anxiety. Experimental anxiety in CRF transgenic mice is reversed by central administration of a CRF receptor antagonist, α -helical CRF (9-41) [75]. Thus, CRF transgenic mice may provide a valuable tool for investigating the over-production of CRF not only in the HPA axis but extrahypothalamic systems as well. This model lends support to the hypothesis that extrahypothalamic CRF plays an important role in behavioral responses to stressors. Moreover, the persistent anxiogenic state exhibited spontaneously by CRF overexpressing mice provides a natural complement to the endocrine and behavioral hyperreactivity following long-term, chronic stressor application, but one in which the disturbances in homeostasis can be attributed largely and selectively to CRF dysregulation. Learning impairment [76] and the absence of sexual receptivity among females [77] are additional maladaptive consequences of the anxiogenic-like behavioral profile induced by CRF overabundance in CRF transgenic mice.

Non-Peptide CRF₁ Receptor Antagonists

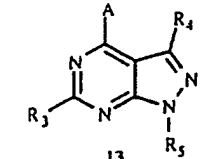
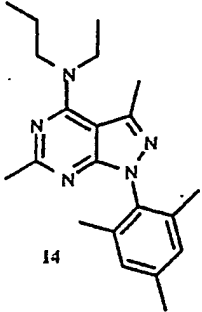
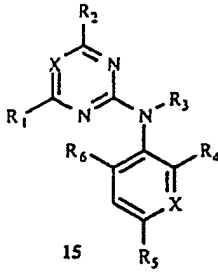
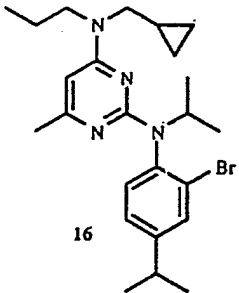
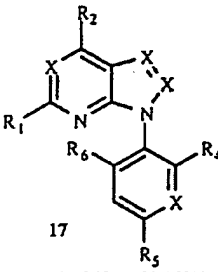
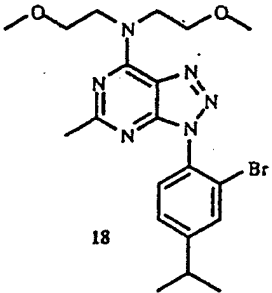
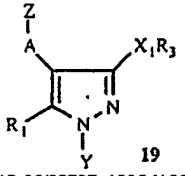
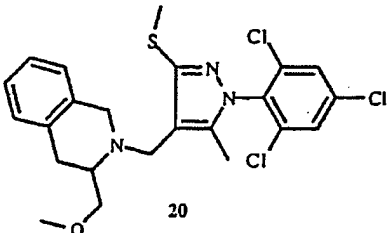
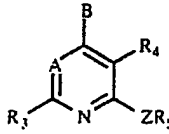
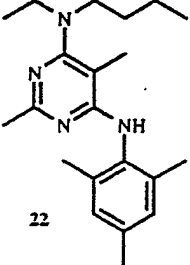
A number of small molecule CRF₁ receptor antagonists have been reported since our review in 1995 [78]. The SAR published on antagonists will be reviewed and patent as well as patent applications will be summarized. Most of the antagonists reported have been cited in patents and patent applications. Table 1 summarizes over 40 patent applications and US patents. Interestingly, most of the CRF₁ antagonists can be envisioned to fit pharmacophore models published [79] and presented [80]. Most of the structures in Table 1 are consistent with this model and contain a phenyl, phenoxy or anilino ring attached to a heteroaryl ring with a bulky alkylamino or alkoxy side chain. The orientation of the key nitrogen in the heteroaryl ring to the phenyl, phenoxy or anilino ring is critical for binding of the molecule to the CRF₁ receptor.

A series of oxopyrazoline thiocyanates, represented by 2, were the first small molecule CRF receptor antagonists reported [81]. The molecules inhibit CRF binding to rat cerebral cortex membrane and CRF-stimulated adenylate cyclase with IC₅₀ values in the 3 to 70 μ M range. Sanofi claimed a series of thiazoles as CRF receptor antagonists without reporting K_i values for the compounds [82]. The SAR for several of the thiazoles was subsequently presented [83]. As seen in Table 2, the importance of the dicyclopropylmethylamino side chain on thiazole 4 (K_i = 10 nM) is apparent from the substantial decrease in binding affinity with the monocyclopropyl analog (115) (K_i = 370 nM), the benzyldryl analog (116) (K_i = 640 nM) and particularly with the "di-open chain" diisopropylmethyl analog (117) (K_i > 10 μ M). The 5-methyl group on the thiazole ring was postulated to play an important role for obtaining tight binding to the CRF₁ receptor by forcing the two aromatic rings to be orthogonal [79]. This hypothesis is consistent with the nearly 100-fold loss in binding for 118 (K_i = 860 nM) vs. 4 and the complete lack of binding to the CRF receptor of the tricyclic analog 119. It is interesting to note that the 2,6-dichloro analog 120 inhibits the binding of CRF to CRF₁ receptors with a K_i in the micromolar range while the 4-chloro analog 121 inhibits the binding of CRF to its receptor with equal potency as the 2,4-dichloro analog 4. These observations were utilized to design a series of small molecule pyrimidine-based CRF₁ selective antagonists (Table 3) with the most potent analogs demonstrating K_i values in the 2 nM range [79]. After a number of changes in the core heterocyclic portion of 4 (see first reported change [84]) and obtaining several inactive series (for example see [79] triazine 122 was screened for activity and found to inhibit the binding of CRF to the CRF₁ receptor with a K_i = 2,100 nM. Elimination of the dicyclopropylmethyl group, while maintaining binding activity was a significant step toward obtaining acid stable [85] novel antagonists. Using a solution phase robotics-driven method for organic synthesis termed Rapid Microscale Synthesis (RMS) [86], triazine 123 (K_i = 57 nM) was synthesized. Further manipulations around the series by conversion of the triazine ring to a pyrimidine ring (initially 124) highlighted the importance of the position of the nitrogens in the pyrimidine ring (see 124 and 126 vs. 125) for binding affinity in the low nanomolar range. Chlorine or a methyl group at the 5-position of 126 led to the most active series represented by 127, 128 and NBI 27,914 (129). Subsequently, Sanofi [87] published a world patent application where the dicyclopropyl groups in compounds similar to 4 were substituted with 2-naphthyl and 3-quinoline groups (e.g. 49).

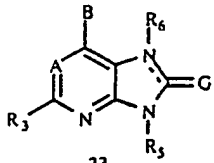
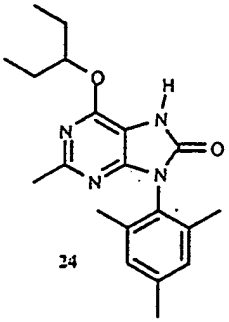
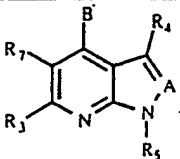
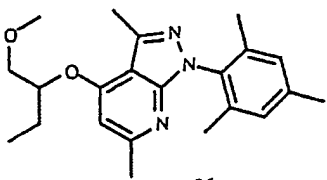
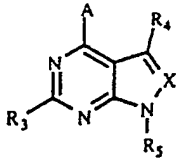
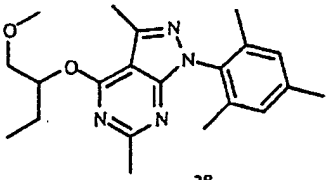
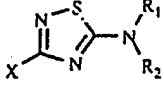
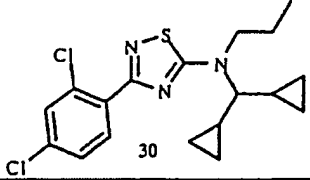
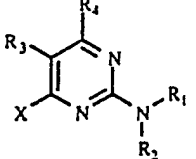
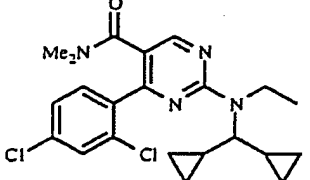
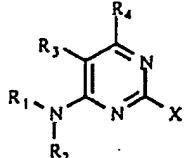
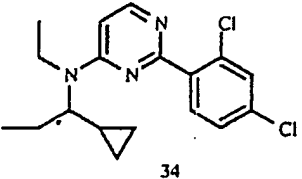
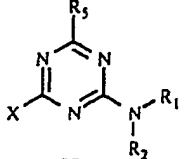
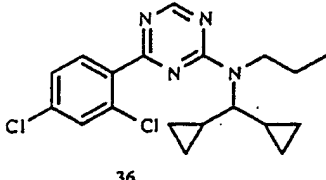
Table 1. Summary of Patent and Patent Applications Claiming CRF Receptor Antagonists

Company	Generic Structure	Example
Nova	 <p>1 US 5,063,245, 1991 [81]</p>	 <p>2</p>
Sanofi	 <p>3 EP 0 576 350, 1993 [82]</p>	 <p>4</p>
Pfizer	 <p>5 WO 94/13643, 1994 [97]</p>	 <p>6</p>
Pfizer	 <p>7 WO 94/13644, 1994 [95]</p>	 <p>8</p>
Pfizer	 <p>9 WO 94/13661, 1994 [96]</p>	 <p>10</p>
Pfizer	 <p>11 WO 94/13676, 1994 [153]</p>	 <p>12</p>

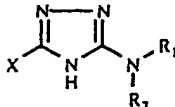
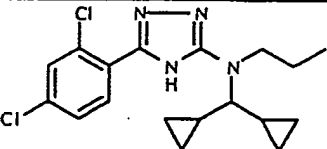
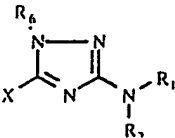
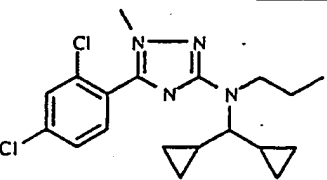
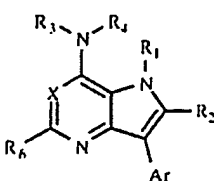
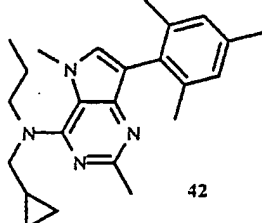
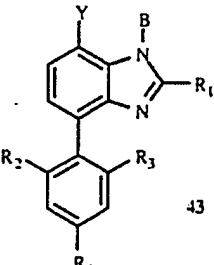
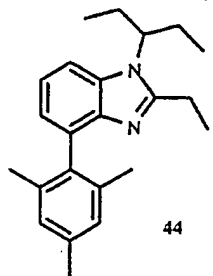
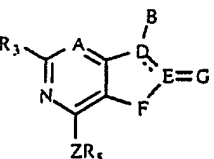
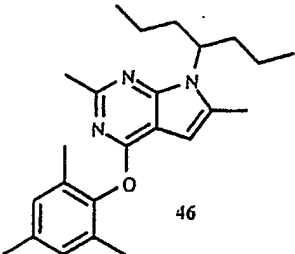
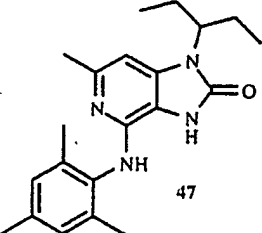
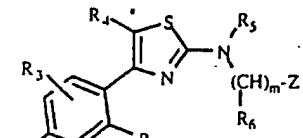
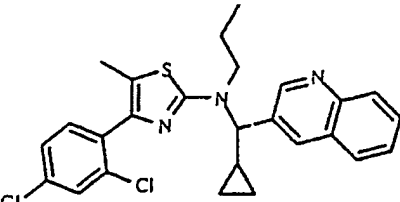
(Table 1). contd....

Company	Generic Structure	Example
Pfizer	 <p>13 WO 94/13677, 1994 [154]</p>	 <p>14</p>
DuPont Merck	 <p>15 WO 95/10506, 1995 [89]</p>	 <p>16</p>
DuPont Merck	 <p>17 WO 95/10506, 1995 [89]</p>	 <p>18</p>
Pfizer	 <p>19 WO 95/33727, 1995 [155]</p>	 <p>20</p>
Pfizer	 <p>21 WO 95/33750, 1995 [88]</p>	 <p>22</p>

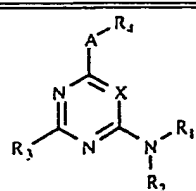
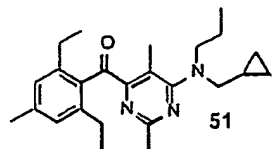
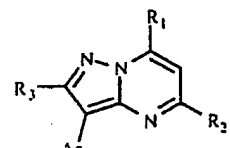
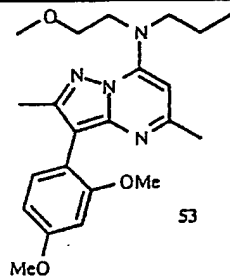
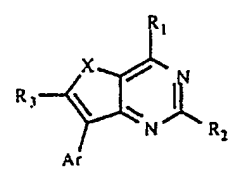
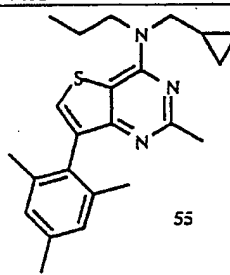
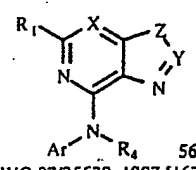
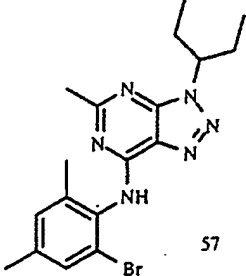
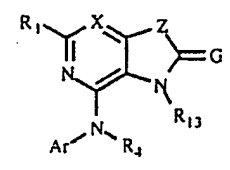
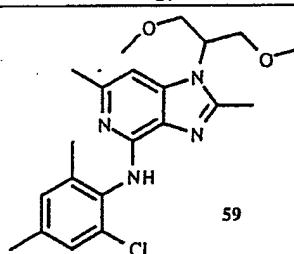
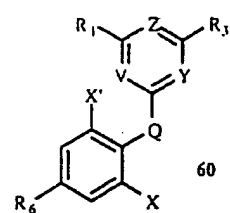
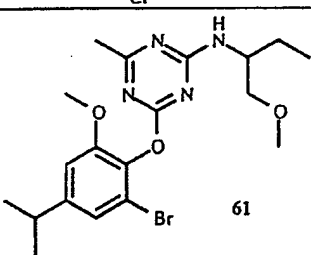
(Table I). contd....

Company	Generic Structure	Example
Pfizer	 <p>23 WO 95/33750, 1995 [88]</p>	 <p>24</p>
Pfizer	 <p>25 WO 95/34563, 1995 [156]</p>	 <p>26</p>
Pfizer	 <p>27 EP 0 729 758, 1996 [157]</p>	 <p>28</p>
Neurocrine	 <p>29 WO 96/39400, 1996 [83]</p>	 <p>30</p>
Neurocrine	 <p>31 WO 96/39400, 1996[83]</p>	 <p>32</p>
Neurocrine	 <p>33 WO 96/39400, 1996[83]</p>	 <p>34</p>
Neurocrine	 <p>35 WO 96/39400, 1996[83]</p>	 <p>36</p>

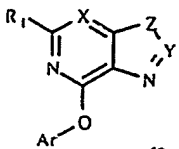
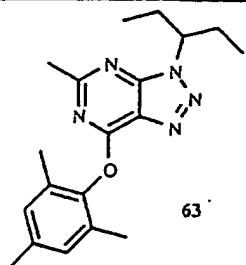
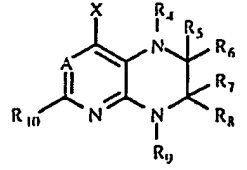
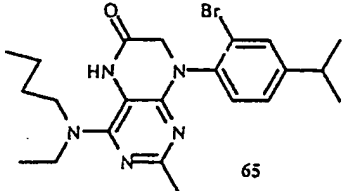
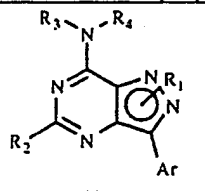
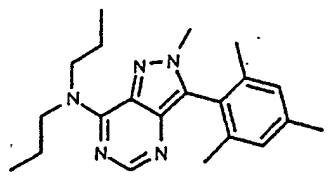
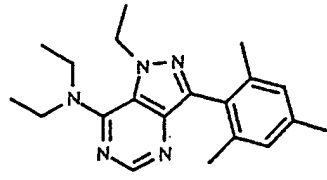
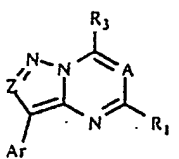
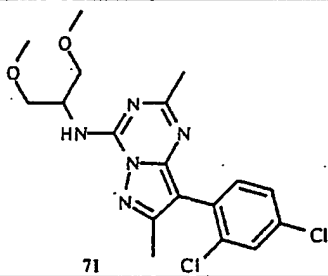
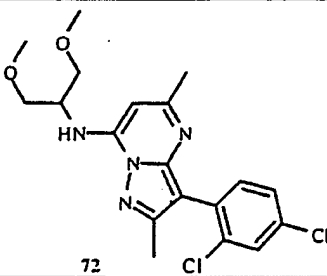
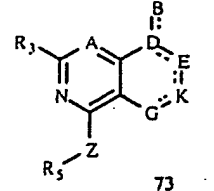
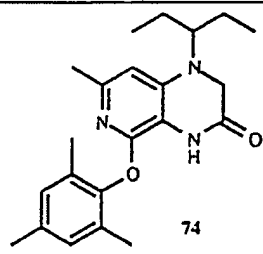
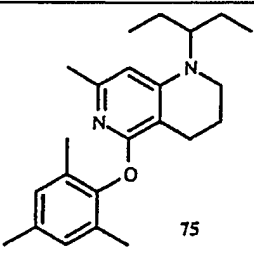
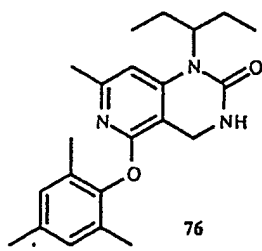
(Table 1), contd....

Company	Generic Structure	Example
Neurocrine	 <p>37 WO 96/39400, 1996[83] US 5,795,905, 1998 [158]</p>	 <p>38</p>
Neurocrine	 <p>39 WO 96/39400, 1996[83] US 5,795,905, 1998 [158]</p>	 <p>40</p>
Neurogen	 <p>41 US 5,664,057, 1997 [102]</p>	 <p>42</p>
Pfizer	 <p>43 EP 0 812 831, 1997 [159]</p>	 <p>44</p>
Pfizer	 <p>45 EP 0 778 277, 1997 [160]</p>	 <p>46</p>  <p>47</p>
Sanofi	 <p>48 WO 97/00868, 1997 [87]</p>	 <p>49</p>

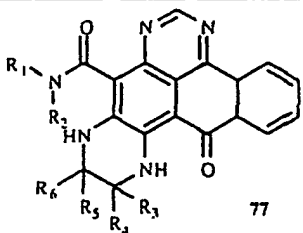
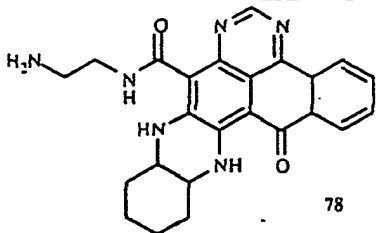
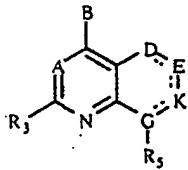
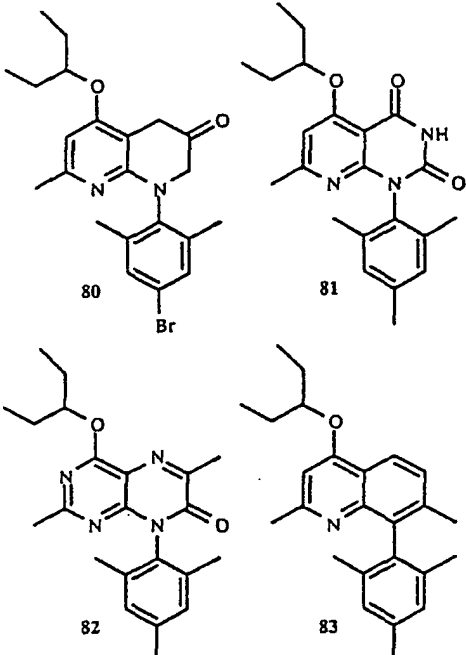
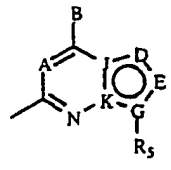
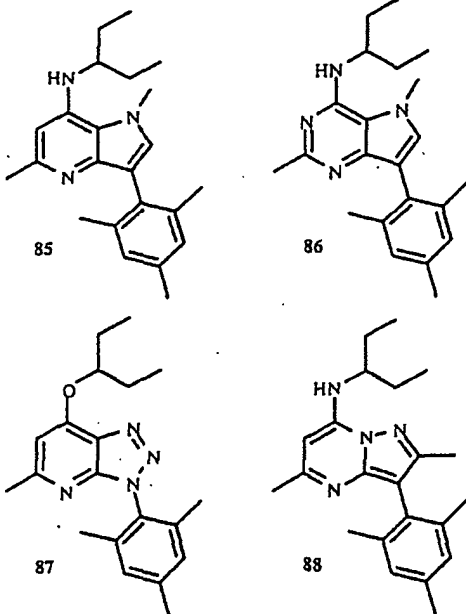
(Table 1). contd....

Company	Generic Structure	Example
Janssen / Neurocrine	 <p>50 WO 97/14684, 1997 [161]</p>	 <p>51</p>
Janssen / Neurocrine	 <p>52 WO 97/29109, 1997 [103]</p>	 <p>53</p>
Janssen / Neurocrine	 <p>54 WO 97/29110, 1997 [104]</p>	 <p>55</p>
Du Pont Merck	 <p>56 WO 97/35539, 1997 [162]</p>	 <p>57</p>
Du Pont Merck	 <p>58 WO 97/35539, 1997 [162]</p>	 <p>59</p>
Du Pont Merck	 <p>60 WO 97/35580, 1997 [163]</p>	 <p>61</p>

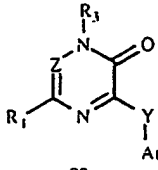
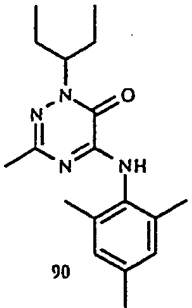
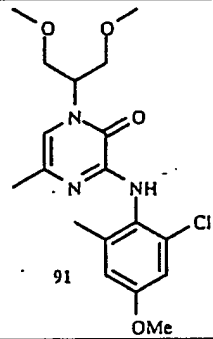
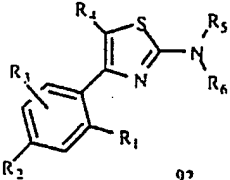
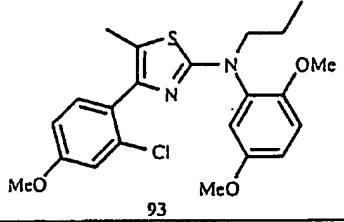
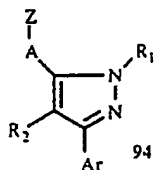
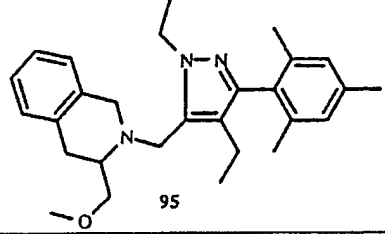
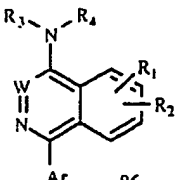
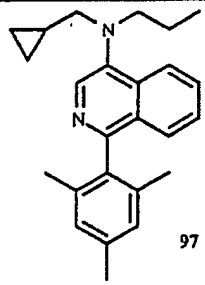
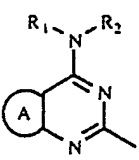
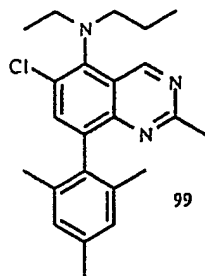
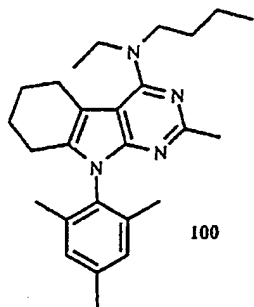
(Table 1). contd....

Company	Generic Structure	Example
Du Pont Merck	 <p>62 WO 97/35846, 1997 [164]</p>	 <p>63</p>
Du Pont Merck	 <p>64 WO 97/44038, 1997 [165]</p>	 <p>65</p>
Neurogen	 <p>66 US 5,723,608, 1998 [166]</p>	 <p>67</p>  <p>68</p>
Du Pont Merck	 <p>70 WO 98/03510, 1998 [105]</p>	 <p>71</p>  <p>72</p>
Pfizer	 <p>73 WO 98/05661, 1998 [167]</p>	 <p>74</p>  <p>75</p>  <p>76</p>

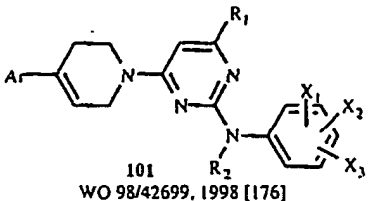
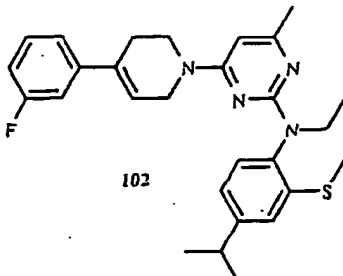
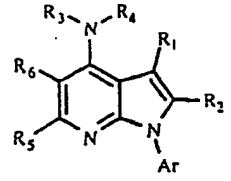
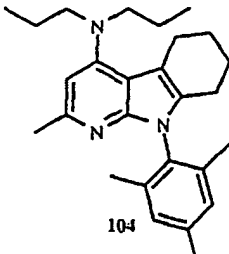
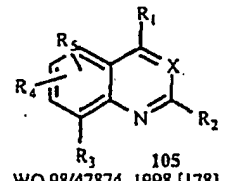
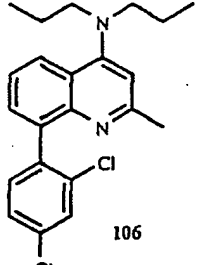
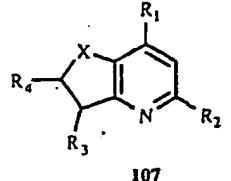
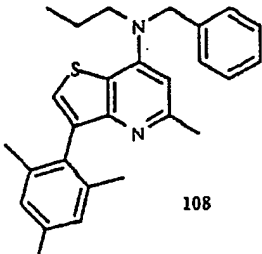
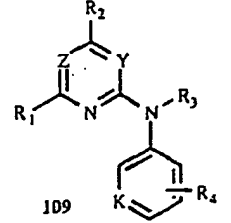
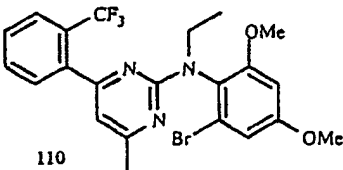
(Table I), contd.

Company	Generic Structure	Example
Alnax	 <p>77</p> <p>WO 98/08821, 1998 [168] US 5,861,398, 1998 [169]</p>	 <p>78</p>
Pfizer	 <p>79</p> <p>WO 98/08846, 1998 [170]</p>	 <p>80 81 82 83</p>
Pfizer	 <p>84</p> <p>WO 98/08847, 1998 [106]</p>	 <p>85 86 87 88</p>

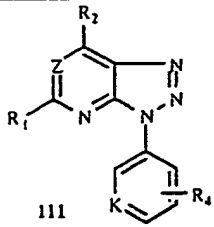
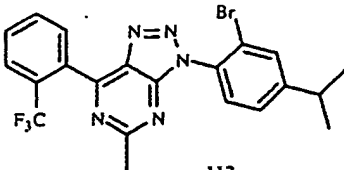
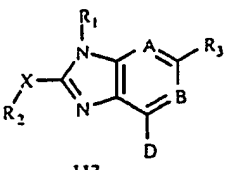
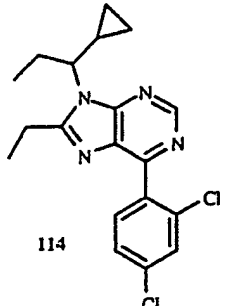
(Table 1), contd....

Company	Generic Structure	Example
Du Pont Merck	 <p>89 WO 98/11075, 1998 [171]</p>	 <p>90</p>  <p>91</p>
Sanofi	 <p>92 WO 98/15543, 1998 [172]</p>	 <p>93</p>
Neurogen	 <p>94 WO 98/21200, 1998 [173]</p>	 <p>95</p>
Neurogen	 <p>96 WO 98/27066, 1998 [174]</p>	 <p>97</p>
Yoshitomi	 <p>98 WO 98/29397, 1998 [175]</p>	 <p>99</p>  <p>100</p>

(Table I). contd....

Company	Generic Structure	Example
Taisho	 <p>101 WO 98/42699, 1998 [176]</p>	 <p>102</p>
Neurogen	 <p>103 WO 98/45295, 1998 [177]</p>	 <p>104</p>
Janssen / Neurocrine	 <p>105 WO 98/47874, 1998 [178]</p>	 <p>106</p>
Janssen / Neurocrine	 <p>107 WO 98/47903, 1998 [179]</p>	 <p>108</p>
Du Pont	 <p>109 WO 99/01439, 1999 [180]</p>	 <p>110</p>

(Table 1). contd....

Company	Generic Structure	Example
Du Pont	 <p>111 WO 99/01439, 1999 [180]</p>	 <p>112</p>
Du Pont	 <p>113 WO 99/01454, 1999 [181]</p>	 <p>114</p>

It is interesting to note the isomeric relationship of the pyrimidines synthesized by Pfizer [88] and DuPont Merck [89] (see 16 and 22) obtained by apparently optimizing two separate lead molecules. In addition, the compounds in Table 3, synthesized at Neurocrine [90] were obtained by a third approach outlined above. A patent application submitted by Neurocrine on these compounds was abandoned after publication of the Pfizer patent application covering 22 and analogs. Du Pont Merck's original screening lead, 2-bromo-N-methylanilinopyrimidine (131) ($K_i = 5700$ nM), was synthesized for agricultural targets [91]. The SAR and structural changes that led to their most potent compound (140) are outlined in Table 4. In contrast to the 4-anilinopyrimidines (see 127 vs. 130, Table 3), substitution of the anilino proton in the Du Pont 2-anilino series with an alkyl group led to potent CRF receptor antagonists (see 132 vs. 133). N-Ethyl substitution was optimal (compare 133 with 134) and 4-substitution on the phenyl ring was critical for potent binding. The 4-isopropyl substituent on the phenyl ring was reported to be optimal (compare 135 with 140). A halogen or a bioisostere (e.g. 136) in the 2-position of the phenyl ring was reported to be essential for potent antagonists and 2,4,6-trisubstituted aryl rings likewise provided potent antagonists. The anilinopyrimidines were highly lipophilic (clog P 6.0-7.5) viscous oils and showed only moderate bioavailability [91] until an amino group was substituted at the 4-position of the pyrimidine ring (e.g. 137). The addition of an alternate polar hydroxy group at the 4-position resulted in complete loss of activity (e.g. 138). Substitution of an additional nitrogen in the pyrimidine ring provided triazines (139) that were inhibitors of comparable potency to the corresponding pyrimidines but with the potential advantage of greater polarity [92]. The triazine nucleus also assisted in increasing bioavailability and provided crystalline free bases that formed stable water-soluble salts. Fused ring triazolopyrimidines (141 and 142), triazolopyridines (143 and 144) and purines (145) (see Table 5) are potent antagonists and support the hypothesis that the N-ethyl substituted analogs in Table 4 provide optimal activity when the ethyl group is coplanar with the pyrimidine or triazine ring [91, 93].

In contrast to the SAR data presented by Du Pont Merck on their pyrimidines and triazines (Table 4) [91, 92] and published by Neurocrine [79, 86] (Table 3), very limited CRF receptor binding data has been published on the pyrimidines and related pyridines claimed in a Pfizer patent application [88]. The compounds specifically cited and the preferred compounds claimed by Pfizer are predominantly 2-anilino-, 2-phenoxy- and 2-thiophenoxypyridines as well as a limited number of related pyrimidines (146-148) [88, 94].

In addition to the aforementioned pyridines and pyrimidines, Pfizer claimed a series of pyrazoles in three separate patent applications [95-97]. Compounds 149 and 150 represent these series. (See previous review for brief overview [98]) No biological data or SAR has been published on these compounds. However, compounds 149-153 were synthesized in these laboratories and the K_i values generated are included in Table 7.

Pfizer reported the biological profile of CP-154,526 (12) (Table 8), a prototypic CRF₁ receptor antagonist [99], with potency similar to NB1-27914 (129). In addition to demonstrating high affinity binding to CRF receptors, $K_i = 1.4$ nM (rat cortex) and the blockade of CRF-stimulated adenylate cyclase in membranes prepared from rat cortex, CP-154,526 antagonizes the stimulatory effects of exogenous CRF on plasma ACTH, locus coeruleus neuronal firing and startle response amplitude. The compound demonstrated potential anxiolytic activity in a fear-potentiated startle paradigm [99]. Very recently, CP-154,526 was reported to be orally active in a rat model of anxiolytic-like effects. In addition, the binding affinities of close analogs of CP-154,526 were published providing some insight into the SAR [100]. It should be noted that Webster [101] published the biological profile on a close analog of CP 154,526 termed antalarmin (154). In the related pyrrolo[3,2-d]pyrimidine series, Neurogen [102] reported compound 155 to have a K_i value of 110 nM. However, a methyl group is not present at the 2-position in this structure which would be consistent with the lower binding affinity.

Table 2. Inhibition (K_i) Values for Selected Thiazoles with CRF Receptors

No.	Structure	K_i (nM)
4		10
115		370
116		640
117		>10,000
118		850
119		>10,000
120		2500
121		10

Table 3. Inhibition (K_i) Values for Selected 2-Anilino-4-aminotriazines and -pyrimidines with CRF Receptors

No.	Structure	K_i (nM)
122		2100
123		57
124		70
125		>10,000
126		30
127		2.3
128		2.5
129		1.7
130		150

Table 4. Inhibition (KI) Values for Selected 2-Anilino- and 2-N-Alkylanilino-pyrimidines with CRF Receptors

No.	Structure	K _i (nM)
131		5700
132		>10,000
133		46
134		96
135		18
136		9
137		8
138		>10,000

(Table 4). contd....

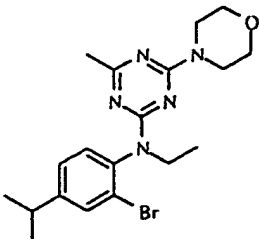
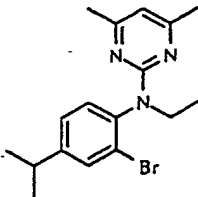
No.	Structure	K _i (nM)
139		70
140		5

Table 5. Inhibition (K_i) Values for Selected Triazolopyrimidines, Triazolopyridines and Purines with CRF Receptors

No.	Structure	K _i (nM)
141		3.8
142		1.9
143		4.0

(Table 5), contd....

No.	Structure	K _i (nM)
144		>10,000
145		4.6

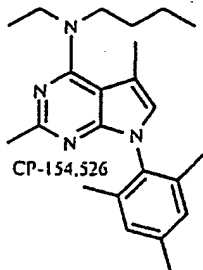
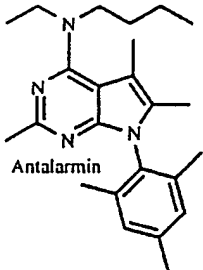
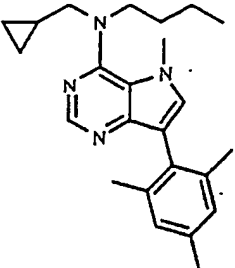
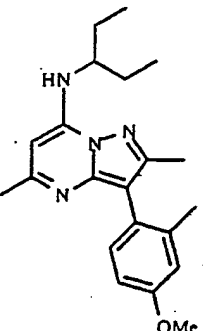
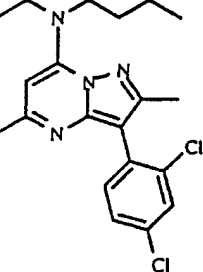
Table 6. Inhibition (K_i) Values for a Selected 4-Benzyl- 4-Phenoxy- and 4-Anilino-pyrimidine with CRF Receptors

No.	Structure	K _i (nM)
146		unreported
147		unreported
148		70

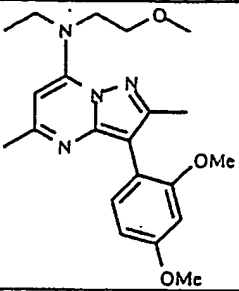
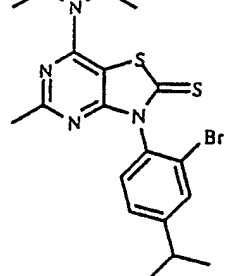
Table 7. Inhibition (K_i) Values for Selected N-Arylpyrazoles with CRF Receptors

No.	Structure	K _i (nM)
149		15
150		11
151		4400
152		250
153		1500

Table 8. Inhibitor (K_i) Values for CP-154,526, Antalarmin, Isomeric Pyrrolopyrimidines, and a Thiazolopyrimidine with CRF Receptors

No.	Structure	K _i (nM)
12	 CP-154,526	2.7
154	 Antalarmin	2.0
155		110
156	 OMe	1.0
157	 Cl Cl	5

(Table 8), contd....

No.	Structure	K _i (nM)
158	 OMe OMe	2.8
159	 Br	4.1

In the past two years almost one half the patent and patent applications have appeared (see Table 1). One series that has received considerable attention by Neurocrine [103, 104], DuPont [105] (see 72), Pfizer [106] (see 88) and Park Davis [107] is the pyrrolo[1,5-a]pyrimidines. The first patent application to appear was published by Neurocrine. Subsequently, DuPont and Pfizer published patent applications. Du Pont presented the pharmacokinetics in dog for pyrrolopyrimidine 156 [108]. In addition, Park Davis presented a poster [109] and published a SAR study around their most potent analog (157) [107]. Neurocrine recently presented the SAR on several of the pyrrolopyrimidines represented by 158 [110]. The discovery of the same series by four separate research groups illustrates the narrow range of small organic molecules that have been found to date that demonstrate high affinity for the CRF₁ receptor. Another example is 8-oxopurines (e.g. 24) presented by DuPont Merck [111] and apparently claimed by both a Pfizer patent application [88] and a DuPont Merck patent application [89]. A similar series, the thiazolo[4,5-b]pyrimidine thiones and -ones, represented by 159, was recently published by Du Pont [112] and is claimed under generic structure 17 (see Table 1). The corresponding 8-one analog of 159 is a slightly less potent CRF₁ receptor antagonist (K_i = 9.4 nM). Another interesting series of antagonists, the N-aryl aminotriazolopyrimidines, represented by 59 (K_i = 4.6 nM) was reported to have good oral bioavailability in dog [113]. Taisho Pharmaceuticals presented a series of 2-anilino-4-aminopyrimidines recently (see 102 in Table 1) [114]. Compound 102 is protected by generic structure 101 and is apparently not covered by earlier 2-anilino-4-amino pyrimidine patents on CRF receptor antagonists. No reports have appeared on the series patented by Alanex (see 77 and 78). However, it is interesting to speculate that this series has activity at the CRF₂ receptor. Alternatively, these structures may provide a starting point for unique CRF₁ receptor antagonists. It is expected that other novel classes of CRF₁ receptor antagonists will

continue to be reported and that diverse structures will be found that bind to the CRF₁ receptor.

Efficacy of CRF₁ Receptor Antagonists in Animal Models of Neuropsychiatric Disorders

Until such time as clinical psychopharmacological data are available for human administration of CRF receptor antagonists, selection of human psychiatric and medical disorders for examination of therapeutic benefit of CRF receptor antagonists will be guided by preclinical, animal model research using CRF family ligands (Table 9). Administration of CRF into the central nervous system produces stress-like activation [115] in a variety of animal behavioral models (Table 10). CRF administered intracerebroventricularly produces increases in locomotor activity, rearing and grooming when rats are tested in a familiar environment [116-118]. This activation is not observed following systemic administration of CRF and is not blocked by hypophysectomy or pretreatment with dexamethasone, suggesting that this effect of CRF is mediated by actions in the central nervous system independent of the pituitary adrenal axis [119-121]. The profile of the behavioral effects of exogenously administered CRF changes dramatically when the animals are exposed to a more stressful environment. The same intracerebroventricular doses that produced marked behavioral activation in a familiar environment produce behavioral inhibition in a novel, presumably stressful environment. Rodents pretreated with CRF show decreases in behavior in an open field [116, 122], in an open field with food [123], in a multicompartment chamber [124], and in an elevated plus maze [125]. CRF produces a proconflict effect in a Geller-Seifter conflict test [126] and in the social interaction test [127]. CRF also enhances the acoustic startle response [128], increases conditioned fear in a conditioned suppression test [129], and enhances stress-induced freezing behavior [130]. Thus, exogenously administered CRF produces a behavioral activation and enhances behavioral responses to stress [49, 131].

A more compelling approach to the question of the role of endogenous CRF in behavioral responses to stress is the demonstration of anti-stress actions of CRF receptor antagonists (Table 10). Evidence using competitive CRF receptor antagonist peptides, α -helical CRF(9-41) and d-Phe CRF(12-41), has provided support for the hypothesis that brain CRF systems play a role in

mediating behavioral responses to stress. In early work, α -helical CRF(9-41) injected centrally was shown to partially reverse the attenuation of feeding induced by stress in rats [132]. Subsequently, α -helical CRF(9-41) was shown to attenuate stress-induced fighting in rats [133]. In mice, α -helical CRF(9-41) reversed the suppression in exploratory behavior produced by restraint stress [134], and in rats α -helical CRF(9-41) produced a more rapid emergence from a small dark enclosure into a large open field and more exploration of the unfamiliar open field [122]. In a more recent study, α -helical CRF(9-41) reversed the decrease in exploration of the open arms of an elevated plus maze caused by exposure to a social stressor [135]. These results using peptide CRF receptor antagonists suggest anti-stress efficacy for treatments which reduce activation of CRF systems in brain.

The prototypical CRF Type I receptor (CRF₁), the recently characterized CRF Type II (CRF₂) receptor and the CRF-binding protein (CRF-BP) constitute separate central nervous system substrates for modulating stress-like activation induced by endogenous CRF-like peptides [136, 137]. These CRF receptor subtypes are distributed heterogeneously within the brain [136] thereby suggesting potential functional diversity. For example, widely distributed brain CRF₁ receptors are strongly implicated in emotionality accompanying exposure to environmental stressors [99, 138]. A more discrete septal/hypothalamic distribution [31] and the availability of alternative endogenous ligands [137] suggest an altogether different functional role for CRF₂. However, while new insights have been provided using non-selective CRF₁ / CRF₂ receptor markers such as [¹²⁵I]-sauvagine [30], comprehensive investigation of these receptor systems has been hampered by lack of selective pharmacological ligands for the CRF₂ receptor. For instance, peptide CRF receptor antagonists such as α -helical CRF(9-41) and d-Phe CRF(12-41) compete with relatively high affinity for both CRF receptor subtypes and in addition bind to the CRF binding-protein [139]. In lieu of appropriate antagonist ligands, an alternative approach for studying the biological significance of specific receptor subtypes involves using antisense oligonucleotides to selectively target a particular receptor population. For example, such an approach has been employed extensively to elucidate the behavioral and physiological significance of a variety of neurotransmitter systems [140]. Accordingly, recent studies examined the impact of CRF₁ and CRF₂ antisense oligonucleotides on behavioral and endocrine indices known to be sensitive to CRF receptor activation [141]. The

Table 9. Potential Clinical Targets for CRF Type I Receptor Antagonists

Disorder	Evidence for CRF ₁ Receptor Involvement	CRF ₁ Receptor Bearing Target Tissue	References
Depression	CRF high in CSF of depressed patients; CRF ₁ receptor antagonist is active in animal model of depression	Central Nervous System	[41, 142]
Anxiety	CRF high in CSF of anxious patients; CRF ₁ receptor antagonist is anxiolytic in animal models of anxiety	Central Nervous System	[39, 115]
Substance Abuse	CRF high in CSF of alcoholic patients; alcohol withdrawal signs diminished in CRF ₁ receptor knockout mice; CRF ₁ receptor antagonist blocks stress-induced reinstatement of cocaine/heroin use	Central Nervous System	[62, 146, 182]
Seizures	CRF ₁ receptor antagonist blunts limbic seizures in infant rats	Central Nervous System	[183]
Inflammation	CRF ₁ receptor antagonist blocks increased vascular permeability in rats	Skin	[11]
Pre-Term Labor	CRF ₁ receptor antagonist delays onset of parturition in fetal sheep	Central Nervous System, Placenta	[149]

Table 10. Behavioral Effects of CRF Receptor Agonists and Antagonists

Paradigm	CRF Agonists	Peptide CRF ₁ / CRF ₂ Receptor Antagonists	Non-Peptide CRF ₁ Receptor Antagonists
Plus-Maze	Suppress exploration of an unfamiliar environment	Reverse stress- and drug-induced suppression of exploration	Anxiolytic-like activity
Acoustic Startle	Facilitate startle	Block fear-potentiated startle	Block fear-potentiated startle
Conditioned Emotional Response	Induce conditioned fear	Block acquisition of emotional response	ND
Cued Electric Shock	Enhance stress- induced freezing	Attenuate stress- induced freezing	ND
Deprivation-Induced Eating	Decrease food intake	Reverse stress- and drug-induced anorexia	No effect on CRF- induced anorexia
Cue Conditioning	Produce aversion	Weaken drug-induced place aversion	ND
Gastric Motility	Delays gastric emptying	Reverse CRF-induced gastric stasis	No effect on CRF- induced gastric stasis
Learned Helplessness	ND	ND	Reverses shock escape deficit
Drug Reinstatement	ND	Attenuate shock- induced reinstatement	Attenuate shock- induced reinstatement

ND - Not yet determined

principle finding of these studies is that CRF₁, but not CRF₂, receptors appear to mediate select anxiogenic-like behaviors. CRF binding site-selective behavioral effects are not unprecedented since a specific and selective CRF-BP ligand inhibitor was recently shown to alter learning and memory capability without inducing anxiogenic-like behavior on the plus-maze whereas administration of a post-synaptic CRF receptor agonist impacted both functional indices [139]. The putative role of CRF₁ receptor antagonists in mediating arousal, affect and stress-induced changes in behavior has been further documented by several recent reports described below of efficacy of small molecule CRF₁ receptors antagonists in animal models of anxiety and depression [100, 142].

Anxiogenesis and Behavioral Despair

The multiple actions of corticotropin-releasing factor (CRF) on neuroendocrine and behavioral functions can now be examined using new, high affinity, small molecule receptor antagonists which exhibit central activity upon systemic application. In particular, the non-peptidic CRF₁ receptor antagonist, CP-154,526, is reported to exert anxiolytic-like activity in the elevated plus-maze test in rats [143]. Other studies [115] have compared the behavioral effects of the CP-154,526 with those of diazepam and the 5-HT_{1A} receptor partial agonist buspirone in classical animal models of anxiety. Unlike diazepam and buspirone and as expected given negative data with peptide CRF receptor antagonists, CP-154,526 was devoid of significant activity in conflict tests (punished lever pressing and punished drinking tests in rats). In a mouse defense test battery which has been validated for the screening of anxiolytic drugs, diazepam attenuated all defensive reactions of mice confronted with a rat stimulus (i.e. flight, risk assessment and defensive attack) or with a situation associated with this threat (i.e. contextual defense). Buspirone reduced defensive attack and contextual defense, while CP-154,526 affected all defensive behaviors, with the exception of one risk assessment measure. Thus, in mice the anxiolytic-like efficacy of CP-154,526 is superior

to that of the atypical anxiolytic buspirone but is smaller in terms of the magnitude of the effects and the number of indices of anxiety affected than that of diazepam [115]. Anxiolytic-like efficacy of CRF₁ antagonists is also reported using the fear-potentiated startle paradigm [99] and the pentobarbital-induced hypnosis test [144].

Potential antidepressant-like effects of CP-154,526 have also been studied [142] using the learned helplessness procedure, a putative model of depression with documented sensitivity to antidepressant drugs. Rats were exposed to a series of inescapable foot shocks on three consecutive days and tested in a shock-escape procedure on the fourth day. Animals trained to exhibit behavioral despair performed poorly in the shock-escape test compared with control animals not receiving inescapable shocks. Systemically administered CP-154,526 dose-dependently reversed the escape deficit when administered one hour prior to the test session, but had no effect on the performance of control rats not receiving prior exposure to inescapable stress. These data support evidence implicating stress systems in the pathophysiology of depression, and suggest potential efficacy of small molecule CRF receptor antagonists in the treatment of affective disorders [142].

Drug Withdrawal and Cocaine/Heroin Reinstatement

CRF has been implicated in the withdrawal syndromes for a variety of drugs of abuse. These prior studies however were unable to address the subtypes of receptor involved because the tools were nonspecific. Recently however the CRF₁ antagonist, CP-154,526, has been examined, and with this agent, the contribution of the CRF₁ receptor in opiate withdrawal has been demonstrated [145]. This work illustrated reductions in CRF₁ mRNA expression in nucleus accumbens and striatum that were dependent on naltrexone precipitated withdrawal. Additionally administration of the selective CRF₁ antagonist CP-154,526 prior to naltrexone significantly decreased many of the somatic opiate withdrawal signs. Supportive evidence implicating activation of the CRF₁

receptor in drug withdrawal has been recently obtained in CRF₁ receptor knockout mice. Mice with CRF₁ deletion exposed to a forced alcohol-drinking procedure and then tested under withdrawal conditions exhibited reduced anxiety-related behavior both under basal conditions and following alcohol withdrawal than wild-type mice [146]. These results highlight an important role for CRF working through the CRF₁ receptor in the expression of drug withdrawal symptoms and further point to the CRF₁ receptor as a potential target for medication development for treating drug abstinence.

Anti-stress efficacy of CP-154,526 has also been examined [62] in a paradigm of stress-induced relapse to drug seeking in cocaine- and heroin-trained rats. Rats were first trained to self-administer heroin or cocaine and then responding for intravenous administration of drug solution was extinguished by substitution of saline. A footshock stressor reliably reinstated extinguished cocaine- and heroin-taking behavior and retreatment with CP-154,526 significantly attenuated the reinstatement effect of the stressor in both heroin- and cocaine-trained rats. CP-154,526, administered in the absence of the footshock stressor, did not affect extinguished drug seeking. In addition, in a separate experiment, CP-154,526 was shown not to alter high rates of lever pressing for a 10% sucrose solution, suggesting that the suppression of lever pressing in stress-induced reinstatement is not caused by a performance deficit. These results extend previous reports on the role of CRF in reinstatement of drug seeking induced by stressors. These data also suggest that, to the extent that exposure to environmental stressors provoke relapse to drug use in humans, systemically effective CRF₁ antagonists have utility in the treatment of relapse to drug use [62].

Hyperexcitability, Seizures and Convulsions

Recent evidence implicates CRF as a key triggering mechanism for seizure generation in the developing brain. Stress activates expression of the CRF gene in several limbic regions and CRF-expressing neurons are strategically localized in the immature rat hippocampus, in which this neuropeptide increases the excitability of pyramidal cells *in vitro*. Indeed activation of CRF receptors, maximally expressed in hippocampus and amygdala during the developmental period which is characterized by peak susceptibility to stress-induced convulsions, produces severe, age-dependent seizures. Both of the characterized members of the CRF receptor family (CRF₁ and CRF₂), are found in the amygdala, site of origin of CRF-induced seizures, and may therefore mediate these seizures. To determine which receptor is responsible for the excitatory effects of CRF on limbic neurons, a selective, non-peptide CRF₁ antagonist was tested for its ability to abolish the seizures, in comparison to non-selective inhibitory analogues of CRF. Pretreatment with NBI 27914 increased the latency and decreased the duration of CRF-induced seizures in a dose-dependent manner. Higher doses of NBI 27914 blocked the behavioral seizures and prevented epileptic discharges in concurrent electroencephalograms recorded from the amygdala. Urocortin, a novel peptide activating both types of CRF receptors *in vitro*, but with preferential affinity for CRF₂ receptors *in vivo*, produced seizures with a lower potency than CRF. These limbic seizures, indistinguishable from those induced by CRF, were abolished by pretreatment with NBI 27914, consistent with their dependence on CRF₁ activation. Thus, converging data indicate that activation of expression of CRF constitutes an important mechanism for generating developmentally

regulated, triggered seizures, with considerable clinical relevance [147].

Potential Utility of CRF Receptor Antagonists for Somatic Disorders

Inflammation

As in stress, intracerebroventricular administration of CRF suppresses the immune system indirectly, via glucocorticoid and/or sympathetic system-mediated mechanisms [148]. Chrousos and colleagues demonstrated a direct role of CRF in the inflammatory immune process *in vivo*, by first studying the effect of systemic CRF immunoneutralization in an experimental model of carrageenin-induced aseptic inflammation in Spague-Dawley rats. CRF induces skin mast cell degranulation and increased vascular permeability, a possible explanation for its proinflammatory effects [11]. These investigators also demonstrated the presence of CRF mRNA and CRF receptors in inflammatory cells and identified the mast cells as a major immune target for CRF. *In vivo* antalarmin significantly inhibited CRF-stimulated ACTH release and carrageenin-induced subcutaneous inflammation in rats. Thus, antalarmin and other related compounds that antagonize CRF at the level of its own receptor have therapeutic potential in some forms of inflammation directly mediated by CRF₁ receptors and promise to enhance our understanding of the many roles of CRF in immune/inflammatory reactions [148]. These results have implications for the pathophysiology and possible therapy of skin disorders, such as atopic dermatitis, eczema, psoriasis, and urticaria, which are exacerbated or precipitated by stress.

Parturition

A CRF₁ antagonist is reported to delay parturition in sheep [149]. CRF can stimulate the fetal release of ACTH to produce a cortisol surge which leads to the onset of parturition. The hypothesis that fetal CRF is a primary factor in the onset of parturition in sheep has been tested using the CRF₁ receptor antagonist, antalarmin, to block the endogenous action of CRF. Pregnant ewes were cannulated at 130-135 days of gestation for the purpose of sampling maternal and fetal blood. Animals received infusions into a fetal vein of either vehicle or antalarmin over 10 days. Fetuses infused with vehicle were delivered sooner than antalarmin-infused and the fetal ACTH and cortisol rise, which characterized the vehicle-treated group, did not occur in antalarmin-infused sheep. These data show that CRF₁ antagonism in the fetus can delay the onset of parturition. It supports the hypothesis that hypothalamic CRF drives fetal production of ACTH and is essential for the onset of parturition triggered by a surge in fetal cortisol [149].

Anticipated Side Effects and Endocrine Toxicity Arising From Long-Term Clinical Administration of CRF Receptor Antagonists

Potential non-specific and endocrine effects of chronic administration of a nonpeptide CRF₁ antagonist have been evaluated using measures of pituitary-adrenal function, body weight, and metabolic regulation. Antalarmin, antagonizes CRF₁-mediated effects of CRF, including pituitary ACTH release, stress-

related behaviors, and acute inflammation. Adult male rats were treated twice daily with 20 mg/kg of i.p. antalarmin over 11 days. Antalarmin decreased plasma ACTH and corticosterone concentrations but had no significant effect on body weight, plasma leptin, or blood glucose concentrations or fat cell leptin messenger RNA levels. These results are consistent with another study in which acute administration of the CRF₁ antagonist NBI 27914 did not affect the decrease in food intake induced by i.c.v. infusion of 3 micrograms CRF [150]. Thus chronic administration of a small molecule CRF₁ antagonist does not affect energy balance while reducing adrenocortical function mildly. These results are promising for future uses of such an antagonist in the clinic [151].

Conclusions / Summary

The fact that studies are now demonstrating that the CRF system consists of a family of receptors that differ dramatically in their structures, pharmacological profiles, anatomical localization within the CNS and periphery, suggests that these proteins (within the scope of this system) will be differentially regulated in response to a variety of physiological challenges. Moreover, the identification of novel endogenous ligands for these subtypes and potent small molecule antagonists for the CRF₁ receptor has led not only to the generation of novel hypotheses regarding CRF function, but more importantly has identified a tangible goal of generating selective non-peptide CRF receptor antagonists as therapeutics which can target specific aspects of CRF-controlled behavior and physiology. The recent demonstration that CRF levels are two to three fold higher than basal not only in patients with anxiety and depression, but after addiction to drugs such as cocaine, heroin and even THC [152] demonstrate the key role that CRF and related ligands play in reflecting human behavior.

References

- [1] Guillemin, R.; Rosenberg, B. *Endocrinology* 1955, 57, 599.
- [2] Saffran, M.; Schally, A.V.; Benfey, B.G. *Endocrinology* 1955, 57, 439.
- [3] Vale, W.; Spiess, J.; Rivier, C.; Rivier, J. *Science* 1981, 213, 1394.
- [4] Vaughan, J.; Donaldson, C.; Bittencourt, J.; Perrin, M.H.; Lewis, K.; Sutton, S.; Chan, R.; Turnbull, A.V.; Lovejoy, D.; Rivier, C.; Rivier, J.; Sawchenko, P.E.; Vale, W. *Nature* 1995, 378(6554), 287.
- [5] Roimier, C.; Bernassau, J.-M.; Cambillau, C.; Darbon, H. *Prot Eng* 1993, 6(2), 149.
- [6] Rivier, J.; Rivier, C.; Vale, W. *Science* 1984, 224, 889.
- [7] Petrusz, P.; Merchenthaler, I. The corticotropin-releasing factor system, In: *Neuroendocrinology*, 1992 (Nemeroff, C.B., ed.). CRC Press, Inc., Boca Raton, FL, pp. 129.
- [8] Sawchenko, P.E.; Swanson, L.W. Organization of CRF immunoreactive cells and fibers in the rat brain, immunohistochemical studies, In: *Corticotropin-releasing factor. Basic and clinical studies of a neuropeptide*, 1990 (De Souza, E.B.; Nemeroff, C.B., eds.). CRC Press, Inc., Boca Raton, FL, pp. 29.
- [9] Swanson, L.W.; Sawchenko, P.E.; Rivier, J.; Vale, W.W. *Neuroendocrinology* 1983, 36, 165.
- [10] Owens, M.J.; Nemeroff, C.B. *Pharmacol Rev* 1991, 43(4), 425.
- [11] Theoharides, T.C.; Singh, L.K.; Boucher, W.; Pang, X.; Letourneau, R.; Webster, E.; Chrousos, G. *Endocrinology* 1998, 139(1), 403.
- [12] Singh, L.K.; Boucher, W.; Pang, X.; Letourneau, R.; Seretakis, D.; Green, M.; Theoharides, T.C. *J Pharmacol Exp Ther* 1999, 288(3), 1349.
- [13] Shibasaki, T.; Odagiri, E.; Shizume, K.; Ling, N. *J Clin Endocrinol Metab* 1982, 55, 384.
- [14] Lowry, P.J.; Woods, R.J.; Baigent, S. *Pharmacol Biochem Behav* 1996, 54(1), 305.
- [15] Di Blasio, A.M.; Giraldi, F.P.; Vigano, P.; Petraglia, F.; Vignali, M.; Cavagnini, F. *J Clin Endocrinol Metab* 1997, 82(5), 1594.
- [16] Aguilera, G.; Millan, M.A.; Hauger, R.L.; Catt, K.J. Corticotropin-releasing factor receptors, Distribution in brain, pituitary and peripheral tissues, In: *The hypothalamic-Pituitary-Adrenal Axis Revisited*, 1987 (Ganong, W.F.; Dallman, M.F.; Roberts, J.L., eds.). The New York Academy of Sciences, New York, pp. 48.
- [17] De Souza, E.B. *J Neurosci* 1987, 7, 88.
- [18] De Souza, E.B.; Kuhar, M.J. *Meth Enzymol* 1986, 124, 560.
- [19] De Souza, E.B. Corticotropin-releasing hormone receptors, In: *Handbook of chemical Neuroanatomy - Neuropeptide receptors in the CNS*, Part III, 1992 (Bjorklund, A.; Hokfelt, T.; Kuhar, M.J., eds.). Elsevier, Amsterdam, pp. 145.
- [20] Chang, C.P.; Pearse, R.I.; O'Connell, S.; Rosenfeld, M.G. *Neuron* 1993, 11(6), 1187.
- [21] Chen, R.; Lewis, K.A.; Perrin, M.H.; Vale, W.W. *Proc Natl Acad Sci (USA)* 1993, 90(19), 8967.
- [22] Perrin, M.H.; Donaldson, C.J.; Chen, R.; Lewis, K.A.; Vale, W.W. *Endocrinology* 1993, 133(6), 3058.
- [23] Vita, N.; Laurent, P.; Lefort, S.; Chalon, P.; Lelias, J.M.; Kaghad, M.; Le, F.G.; Coput, D.; Ferrara, P. *FEBS Lett* 1993, 335(1), 1.
- [24] Lovenberg, T.W.; Liaw, C.W.; Grigoriadis, D.E.; Clevenger, W.; Chalmers, D.T.; De Souza, E.B.; Oltersdorf, T. *Proc Natl Acad Sci (USA)* 1995, 92, 836.
- [25] Kishimoto, T.; Pearse II, R.V.; Lin, C.R.; Rosenfeld, M.G. *Proc Natl Acad Sci (USA)* 1995, 92, 1108.
- [26] Perrin, M.; Donaldson, C.; Chen, R.; Blount, A.; Berggren, T.; Bilezikjian, L.; Sawchenko, P.; Vale, W. *Proc Natl Acad Sci (USA)* 1995, 92, 2969.
- [27] Kostich, W.; Chen, A.; Sperle, K.; Horlick, R.A.; Patterson, J.; Largent, B.L. *Soc Neurosci Abstr* 1996, 22(2), 1545.
- [28] Lovenberg, T.W.; Chalmers, D.T.; Liu, C.; De Souza, E.B. *Endocrinology* 1995, 136, 4139.
- [29] Grigoriadis, D.E.; Liaw, C.W.; Oltersdorf, T.; De Souza, E.B. *Soc Neurosci Abstr* 1994, 20, 1345.
- [30] Grigoriadis, D.E.; Liu, X.J.; Vaughn, J.; Palmer, S.F.; True, C.D.; Vale, W.W.; Ling, N.; De Souza, E.B. *Mol Pharmacol* 1996, 50, 679.
- [31] Chalmers, D.T.; Lovenberg, T.W.; De Souza, E.B. *J Neurosci* 1995, 15, 6340.
- [32] De France, J.F. *The Septal Nuclei*, ed., 1976, New York, Plenum.
- [33] Sakanaka, M.; Magari, S.; Shibasaki, T.; Lederis, K. *J Comp Neurol* 1988, 270, 404.
- [34] De Souza, E.B.; Grigoriadis, D.E. Corticotropin-releasing factor, Physiology, pharmacology and role in central nervous system and immune disorders, In: *Psychopharmacology, The Fourth Generation of Progress*, 1994 (Bloom, F.E.; Kupfer, D.J., eds.). Raven Press, New York, pp. 505.

- [35] De Souza, E.B.; Nemeroff, C.B. Corticotropin-releasing factor. Basic and clinical studies of a neuropeptide. CRC Press, Inc., Boca Raton, Florida, 365 (1990).
- [36] Grigoriadis, D.E.; Heroux, J.A.; De Souza, E.B. Characterization and regulation of corticotropin-releasing factor receptors in the central nervous, endocrine and immune systems. In: Corticotropin-Releasing Factor, 1993 (Chadwick, D.J.; Marsh, J.; Ackrill, K., eds.). John Wiley and Sons Ltd., Chichester, West Sussex, pp. 85.
- [37] Birnbaumer, L. *Annu Rev Pharmacol Toxicol* 1990, 30, 675.
- [38] Moreau, J.L.; Kilpatrick, G.; Jenck, F. *Neuroreport* 1997, 8(7), 1697.
- [39] Bremner, J.D.; Licinio, J.; Damell, A.; Krystall, J.H.; Owens, M.J.; Southwick, S.M.; Nemeroff, C.B.; Charney, D.S. *Am J Psychiatry* 1997, 154(5), 624.
- [40] Yehuda, R.; Teicher, M.H.; Levengood, R.A.; Trestman, R.L.; Siever, L.J. *Ann New York Acad Sci* 1994, 746, 378.
- [41] Nemeroff, C.B. *Biol Psychiatry* 1998, 44(7), 517.
- [42] Nemeroff, C.B.; Owens, M.J.; Bissett, G.; Andorn, A.C.; Stanley, M. *Arch Gen Psychiatry* 1988, 45, 577.
- [43] Roy, A.; Pickar, D.; Paul, S.; Doran, A.; Chrousos, G.P.; Gold, P.W. *Am J Psychiatry* 1987, 143, 896.
- [44] Nemeroff, C.B.; Bissett, G.; Akil, H.; Fink, M. *Br J Psychiatry* 1991, 158, 59.
- [45] Grigoriadis, D.E.; Pearsall, D.M.; De Souza, E.B. *Neuropsychopharmacology* 1989, 2, 53.
- [46] Valentino, R.J.; Foote, S.L.; Page, M.E. *Ann New York Acad Sci* 1993, 697, 173.
- [47] Chappell, P.B.; Smith, M.A.; Kilts, C.D.; Bissett, G.; Ritchie, J.; Anderson, C.; Nemeroff, C.B. *J Neurosci* 1986, 6, 2908.
- [48] Valentino, R.J.; Foote, S.L.; Aston, J.G. *Brain Res* 1990, 270(363), 363.
- [49] Koob, G.F.; Heinrichs, S.C.; Menzaghi, F.; Merlo Pich, E.; Britton, K.T. *Seminars in Neuroscience* 1994, 6, 221.
- [50] Roy-Byrne, P.P.; Uhde, T.; Post, R.; Gallucci, W.; Chrousos, G.P.; Gold, P.W. *Am J Psychiatry* 1986, 143, 896.
- [51] Jones, D.N.; Kortikaas, R.; Slade, P.D.; Middlemiss, D.N.; Hagan, J.J. *Psychopharmacol* 1998, 138(2), 124.
- [52] Smith, M.A.; Weiss, S.R.B.; Berry, R.L.; Zhang, L.-X.; Clark, M.; Massenburg, G.; Post, R.M. *Brain Res* 1997, 745, 248.
- [53] APA. Diagnostic and Statistical Manual of Mental Disorders - Fourth Edition. (DSM-IV), ed., 1994, Washington DC, American Psychiatric Association.
- [54] Owens, M.J.; Vargas, M.A.; Knight, D.L.; Nemeroff, C.B. *J Pharmacol Exp Ther* 1991, 258, 349.
- [55] Calogero, A.E. *Ann N Y Acad Sci* 1995, 771, 31.
- [56] Samyay, Z.; Biró, É.; Penke, B.; Telegdy, G. *Brain Res* 1992, 589, 154.
- [57] Rivier, C.; Vale, W. *Brain Res* 1987, 422, 403.
- [58] Fischman, M.W.; Schuster, C.R.; Harano, Y. *Pharmacol Biochem Behav* 1983, 18(123), 123.
- [59] Teoh, S.K.; Samyay, Z.; Mendelson, J.H.; Mello, N.K.; Springer, S.A.; Sholar, J.W.; Wapler, M.; Kuehnle, J.C.; Gelles, H. *J Pharmacol Exp Ther* 1994, 270(3), 1134.
- [60] Berger, S.P.; Hall, S.; Mickalian, J.D.; Reid, M.S.; Crawford, C.A.; Delucchi, K.; Carr, K.; Hall, S. *Lancet* 1996, 347, 504.
- [61] Piazza, P.V.; Le Moal, M. *Annual Reviews of Pharmacology and Toxicology* 1996, 36, 359.
- [62] Shaham, Y.; Leung, S.; Buczak, Y.; J. S. *Psychopharmacol* 1998, 137(2), 184.
- [63] Zhou, Y.; Spangler, R.; LaForge, K.S.; Maggos, C.E.; Ho, A.; Koob, G.F. *J Pharmacol Exp Ther* 1996, 279(1), 351.
- [64] Yang, X.-M.; Gonnard, A.L.; Dunn, A.J.; Goeders, N.E. *Pharmacol Biochem Behav* 1992, 41, 643.
- [65] Shaham, Y.; Rajabi, H.; Stewart, J. *J Neurosci* 1996, 16(5), 1957.
- [66] Goeders, N.E.; Guerin, G.F. *Psychopharmacol* 1994, 114, 63.
- [67] Koob, G.F. *Neuron* 1996, 16, 893.
- [68] Gawin, F.H.; Kleber, H.D. *Arch Gen Psychiatry* 1986, 43, 107.
- [69] Cooney, N.L.; Litt, M.D.; Morse, P.A.; Bauer, L.O.; Gaupp, L. *Journal of Abnormal Psychology* 1997, 106(2), 243.
- [70] Brown, S.A.; Vik, P.W.; McQuaid, J.R.; Patterson, T.L.; Irwin, M.R.; Grant, I. *Journal of Abnormal Psychology* 1990, 99(4), 344.
- [71] Childress, A.R.; Ehrman, R.; McLellan, A.T.; MacRae, J.; Natale, M.; O'Brien, C.P. *Journal of Substance Abuse Treatment* 1994, 11(1), 17.
- [72] Litt, M.D.; Cooney, N.L.; Kadden, R.M.; Gaupp, L. *Addictive Behaviors* 1990, 15(2), 137.
- [73] Tiffany, S.T.; Drobos, D.J. *Addictive Behaviors* 1990, 15(6), 531.
- [74] Stenzel-Poore, M.P.; Duncan, J.E.; Rittenberg, M.B.; Bakke, A.C.; Heinrichs, S.C. *Ann N Y Acad Sci* 1996, 780, 36.
- [75] Stenzel-Poore, M.P.; Heinrichs, S.C.; Rivest, S.; Koob, G.F.; Vale, W.W. *The journal of neuroscience* 1994, 14, 2579.
- [76] Heinrichs, S.C.; Stenzel-Poore, M.P.; Gold, L.H.; Battenberg, E.; Bloom, F.E.; Koob, G.F.; Vale, W.W.; Merlo Pich, E. *Neuroscience* 1996, 74(2), 303.
- [77] Heinrichs, S.C.; Min, H.; Tamraz, S.; Carinouché, M.; Boehme, S.; Vale, W.W. *Psychoneuroendocrinology* 1997, 22(4), 215.
- [78] Lovenberg, T.W.; Grigoriadis, D.E.; Chalmers, D.T.; McCarthy, J.R.; De Souza, E.B. *Current Pharmaceutical Design* 1995, 1, 305.
- [79] Chen, C.; Dagnino Jr., R.; De Souza, E.B.; Grigoriadis, D.E.; Huang, C.Q.; Kim, K.I.; Lui, Z.; Moran, T.; Webb, T.R.; Whitten, J.P.; Xie, Y.F.; McCarthy, J.R. *J Med Chem* 1996, 39, 4358.
- [80] Hodge, C.N.; Aldrich, P.E.; Fernandez, C.; Chorvat, R.J.; Cheesman, R.S.; Christos, T.E.; Arvanitis, A.G.; Scholfield, E.; Krenitsky, P.J.; Gilligan, P.J.; Ciganek, E.; Strucely, P.; Wasserman, Z.R. Anilinopyrimidines as corticotropin-releasing hormone (CRH) antagonists, in 212 ACS National Meeting, American Chemical Society, Orlando FL, MEDI 094 (1996).
- [81] Abreu, M.E.; Rzeszutowski, W.; Kyle, D.J.; Elliot, R.L. Corticotropin-releasing factor antagonism compounds, 1991. US 5,063,245.
- [82] Courtemanche, G.; Gautier, C.; Gully, D.; Roger, P.; Valente, G.; Wermuth, C.G. Dérivés alkylamino ramifiés du thiazole, leurs procédés de préparation et les compositions pharmaceutiques qui les contiennent, 1993, EP 0 576 350 A1.
- [83] McCarthy, J.R.; Whitten, J.P.; Webb, T.R.; Ramphal, J.Y.; Grigoriadis, D.E.; Chen, C.; Huang, C.Q.; Liu, Z.; Xie, Y.F.; Dagnino, R. Amino-substituted thiadiazoles, pyrimidines, triazines or triazoles useful as CRF receptor antagonists, 1996, WO 96/39400.
- [84] McCarthy, J.R.; Chen, C.; De Souza, E.B.; Erickson, P.E.; Grigoriadis, D.E.; Webb, T.R.; Whitten, J.P.; Xie, Y.F. Design and synthesis of potent and selective CRF1 receptor antagonists with the

- aid of rapid microscale synthesis, in *212 ACS National Meeting*, American Chemical Society, Orlando FL, MEDI 196 (1996).
- [85] Carpino, L.A.; H.-G., C.; Ghassemi, S.; Mansour, E.M.E.; Reimer, C.; Warrass, R.; Sadar-Aalae, D.; Turan, G.A.; Imazumi, H.; El-Faham, A.; Ionescu, D.; Ismail, M.; Kowaleski, T.L.; Han, C.H. *Journal of Organic Chemistry* 1995, 60, 7718.
- [86] Whiten, J.P.; Xie, Y.F.; Erickson, P.E.; Webb, T.R.; De Souza, E.B.; Grigoriadis, D.E.; McCarthy, J.R. *J Med Chem* 1996, 39, 4354.
- [87] Gully, D.; Roger, P.; Wermuth, C.G. 4-phenylaminothiazole derivatives, method for preparing same and pharmaceutical compositions containing said derivatives., 1997, WO 97/00868.
- [88] Chen, Y.L. Corticotropin releasing factor antagonists, 1995, WO 95/33750.
- [89] Aldrich, P.E.; Arvanitis, A.G.; Cheeseman, R.S.; Chorvat, R.J.; Christos, T.E.; Gilligan, P.J.; Grigoriadis, D.E.; Hodge, C.N.; Krenitsky, P.J.; Scholfield, E.L.; Tam, S.W.; Wasserman, Z.R. *N-Alkyl-N-arylpyrimidinamines and derivatives thereof*, 1995, WO 95/10506.
- [90] Chen, C.; Dagnino, J. R.; McCarthy, J.R. *Journal of Organic Chemistry* 1995, 60, 8428.
- [91] Arvanitis, A.G.; Aldrich, P.E.; Arnold, C.R.; Bakthavatchalam, R.; Beck, J.P.; Bouchard, P.J.; Cheesman, R.S.; Chidester, D.R.; Chorvat, R.J.; Christos, T.E.; Cocuzza, A.J.; Culp, S.J.; Curry, M.; Fitzgerald, L.W.; Gilligan, P.J.; Hartig, P.R.; He, L.; Hobbs, F.W.; Hodge, C.N.; Krenitsky, P.J.; McCall, D.E.; Rescinito, J.T.; Scholfield, E.L.; Tam, S.W.; Trainor, G.L.; Wasserman, Z.R.; Wilde, J.A.; Yarem, J.A.; Zaczek, R. Anilinopyrimidines as corticotropin-releasing hormone (CRH) antagonists, in *212 ACS National Meeting*, American Chemical Society, Orlando FL, MEDI 194 (1996).
- [92] Christos, T.E.; Arvanitis, A.G.; Beck, J.P.; Cheesman, R.S.; Chorvat, R.J.; Cocuzza, A.J.; Culp, S.J.; Gilligan, P.J.; Hartig, P.R.; Hobbs, F.W.; Klaczewicz, J.; Krenitsky, P.J.; Scholfield, E.L.; Wilde, J.A.; Zaczek, R. Anilinopyrimidines as corticotropin-releasing hormone (CRH) antagonists, in *212 ACS National Meeting*, American Chemical Society, Orlando FL, MEDI 092 (1996).
- [93] Bakthavatchalam, R.; Aldrich, P.E.; Arvanitis, A.G.; Beck, J.P.; Calabrese, J.C.; Cheesman, R.S.; Chorvat, R.J.; Christos, T.E.; Cocuzza, A.J.; Culp, S.J.; Curry, M.; Fitzgerald, L.W.; Gilligan, P.J.; Hobbs, F.W.; Hodge, C.N.; Huang, S.-M.; Krenitsky, P.J.; McCall, D.E.; Rescinito, J.T.; Wasserman, Z.R.; Wilde, J.A.; Wong, N.Y.; Yarem, J.A.; Zaczek, R. Anilinopyrimidines as corticotropin-releasing hormone (CRH) antagonists, in *212 ACS National Meeting*, American Chemical Society, Orlando FL, MEDI 093 (1996).
- [94] Chen, Y.L.; Fossa, A.A. New Uses for corticotropin releasing factor (CRF) antagonists, 1997, EP 0 773 023 A1.
- [95] Bright, G.M. Amino-substituted pyrazoles having CRF antagonist activity, 1994, WO 94/13644.
- [96] Bright, G.M.; Welch, W.M.J. Substituted pyrazoles as CRF antagonists, 1994, WO 94/13661.
- [97] Faraci, W.S.; Welch, W.M.J. Pyrazoles and pyrazolopyrimidines having CRF antagonist activity., 1994, WO 94/13643.
- [98] De Souza, E.B.; Lovenberg, T.W.; Chalmers, D.T.; Grigoriadis, D.E.; Liaw, C.W.; Behan, D.P.; McCarthy, J.R. *Annual Reports in Medicinal Chemistry* 1995, 30, 21.
- [99] Schulz, D.W.; Mansbach, R.S.; Sprouse, J.; Braselton, J.P.; Collins, J.; M., C.; Dunaikis, A.; Faraci, S.; Schmidt, A.W.; Seeger, T.; Seymour, P.; Tingley III, F.D.; Winston, E.N.; Chen, Y.L.; Heym, J. *Proceedings of the National Academy of Sciences USA* 1996, 93, 10477.
- [100] Chen, Y.L.; Mansbach, R.S.; Winter, S.M.; Brooks, E.; Collins, J.; Cornman, M.L.; Dunaikis, A.R.; Faraci, W.S.; Gallaschun, R.J.; Schmidt, A.; Schulz, D.W. *J Med Chem* 1997, 40(11), 1749.
- [101] Webster, E.L.; Lewis, D.B.; Torpy, D.J.; Zachman, E.K.; Rice, K.C.; Chrousos, G.P. *Endocrinology* 1996, 137(12), 5747.
- [102] Yuan, J.; Hutchinson, A. Deazapurine derivatives, a new class of CRF1 specific ligands, 1997, US 5,644,057.
- [103] Chen, C.; Webb, T.R.; McCarthy, J.R.; Wilcoxon, K.M. Pyrazolopyrimidines as CRF receptor antagonists, 1997, WO 97/29109.
- [104] Chen, C.; Webb, T.R.; McCarthy, J.R.; Moran, T.J. Thiophenopyrimidines, 1997, WO 97/29110.
- [105] Arvanitis, A.G.; Chorvat, R.J. Azolo triazines and pyrimidines, 1998, WO 98/03510.
- [106] Chen, Y.L. Substituted 6,5-hetero-bicyclic derivatives, 1998, WO 98/08847.
- [107] Wustrow, D.J.; Capiris, T.; Rubin, R.; Knobelsdorf, J.A.; Akunne, H.; Davis, M.D.; MacKenzie, R.; Pugsley, T.A.; Zoski, K.T.; Heffner, T.G.; Wise, L.D. *Bioorg Med Chem Lett* 1998, 8(16), 2067.
- [108] Gilligan, P.J.; Baldauf, C.; Cocuzza, A.; Chidester, D.; Fitzgerald, L.; Zaczek, R.; Shen, H. Pyrazolo[1,5-a]-pyrimidine CRF antagonists, Synthesis and structure activity., in *216 ACS National Meeting*, American Chemical Society, Boston, MA, 135 (1998).
- [109] Capiris, T.; Wustrow, D.J.; Rubin, M.R.; Knobelsdorf, J.A.; Akunne, H.; Davis, M.D.; MacKenzie, R.; Pugsley, T.A.; Zoski, K.T.; Heffner, T.G.; Wise, L.D. Pyrazolo[1,5-a]pyrimidineCRF-1 receptor antagonists, in *26th National Medicinal Chemistry Symposium*, Omni Richmond Hotel, Richmond VA, D (1998).
- [110] Wilcoxon, K.; Chen, C.; Huang, C.; Haddach, M.; Xie, Y.-F.; Wing, L.; Grigoriadis, D.E.; De Souza, E.B.; McCarthy, J.R. Design and synthesis of NBI 30545 and analogues as potent corticotropin-releasing factor₁ (CRF₁) receptor antagonists, in *217 ACS National Meeting*, Anaheim, CA, MEDI 02 (1999).
- [111] Beck, J.P.; Arvanitis, A.G.; Cocuzza, A.J.; Chidester, D.R.; Curry, M.A.; Rescinito, J.T.; Fitzgerald, L.W.; Zaczek, R.; Calabrese, J.C. 8-oxopurines as CRH receptor antagonists, in *214 ACS National Meeting*, American Chemical Society, Las Vegas, NV, MEDI 094 (1997).
- [112] Beck, J.P.; Curry, M.A.; Folmer, B.K.; Gilligan, P.J.; Robertson, D.W.; Fitzgerald, L.W.; Zaczek, R.; Calabrese, J.C. Thiazolo[4,5-d]pyrimidinethiones and -ones as potent corticotropin-releasing hormone (CRH-R1) receptor antagonists, in *216 ACS National Meeting*, American Chemical Society, Boston, MA, 136 (1998).
- [113] Bakthavatchalam, R.; Arvanitis, A.G.; Gilligan, P.J.; Olson, R.E.; Robertson, D.W.; Trainor, G.L.; Smith, S.C.; Fitzgerald, L.W.; Zaczek, R.; Shen, H.; Christ, D.D. The discovery of DMP 695, an orally active corticotropin-releasing hormone (CRH1) receptor antagonist., in *216 ACS National Meeting*, American Chemical Society, Boston, MA, 134 (1998).
- [114] Nakazato, A.; Okubo, T.; Kumagai, T.; Chaki, S.; Okuyama, S.; Tomisawa, K. Aryl-1,2,3,6-tetrahydropyridinopyrimidine derivatives as CRF1 receptor antagonists, in *216 ACS National Meeting*, American Chemical Society, Boston, MA, 137 (1998).
- [115] Griebel, G.; Perrault, G.; Sanger, D.J. *Psychopharmacol* 1998, 138(1), 55.
- [116] Sutton, R.E.; Koob, G.F.; Le Moal, M.; Rivier, J.; Vale, W. *Nature* 1982, 297, 331.

- [117] Koob, G.F.; Swerdlow, N.; Scoldingson, M. *Neuroendocrinology* 1984, 39, 459.
- [118] Sherman, J.E.; Kalin, N.H. *Pharmacol Biochem Behav* 1987, 26, 699.
- [119] Eaves, M.; Thatcher, B.K.; Rivier, J.; Vale, W.; Koob, G.F. *Peptides* 1985, 6(5), 923.
- [120] Britton, D.R.; Varela, M.; Garcia, A.; Rosenthal, M. *Life Sci* 1986, 38, 211.
- [121] Britton, K.T.; Lee, G.; Dana, R.; Risch, S.C.; Koob, G.F. *Life Sci* 1986, 39, 1281.
- [122] Takahashi, L.K.; Kalin, N.H.; Vandenberg, J.A.; Sherman, J.E. *Behavioral Neurosciences* 1989, 103, 648.
- [123] Britton, D.R.; Koob, G.F.; Rivier, J.; Vale, W. *Life Sci* 1982, 31, 363.
- [124] Berridge, C.W.; Dunn, A.J. *Regul Peptides* 1986, 16, 83.
- [125] Baldwin, H.A.; Rassnick, S.; Rivier, J.; Koob, G.F.; Britton, K.T. *Psychopharmacol* 1991, 103, 227.
- [126] Britton, K.; Morgan, J.; Rivier, J.; Vale, W.; Koob, G.F. *Psychopharmacol* 1985, 86, 170.
- [127] Dunn, A.J.; File, S.E. *Horm Behav* 1987, 21, 193.
- [128] Swerdlow, N.R.; Geyer, M.A.; Vale, W.W.; Koob, G.F. *Psychopharmacol* 1986, 88, 147.
- [129] Cole, B.J.; Koob, G.F. *J Pharmacol Exp Ther* 1988, 247, 902.
- [130] Sherman, J.E.; Kalin, N.E. *Pharmacol Biochem Behav* 1988, 30, 801.
- [131] Dunn, A.J.; Berridge, C.W. *Br Res Rev* 1990, 15, 71.
- [132] Krahn, D.D.; Gosnell, B.A.; Grace, M.; Levine, A.S. *Brain Res Bull* 1986, 17, 285.
- [133] Tazi, A.; Dantzer, R.; Le Moal, M.; Rivier, J.; Vale, W.; Koob, G.F. *Regul Peptides* 1987, 18, 37.
- [134] Berridge, C.W.; Dunn, A.J. *Horm Behav* 1987, 21, 393.
- [135] Heinrichs, S.C.; Merlo Pich, E.; Miczek, K.A.; Britton, K.T.; Koob, G.F. *Brain Res* 1992, 581, 190.
- [136] Chalmers, D.T.; Lovenberg, T.W.; Grigoriadis, D.E.; Behan, D.P.; De Souza, E.B. *Trends Pharmacol Sci* 1996, 17, 166.
- [137] Vaughan, J.; Donaldson, C.; Bittencourt, J.; Lewis, K.; Lovejoy, D.; Black, J.; Rivier, J.; Sawchenko, P.; Vale, W. Characterization of a novel neuropeptide in rat brain related to CRF, in *25th Annual Meeting Of The Neuroscience Society*, San Diego (1995).
- [138] Liebsch, G.; Landgraf, R.; Gerstberger, R.; Probst, J.C.; Wotjak, C.T.; Engelmann, M.; Holsboer, F.; Montkowski, A. *Regul Peptides* 1995, 59, 229.
- [139] Behan, D.P.; Heinrichs, S.C.; Troncoso, J.C.; Liu, X.J.; Kawas, C.H.; Ling, N.; De Souza, E.B. *Nature* 1995, 378, 284.
- [140] Phillips, M.L.; Gyurko, R. *Regul Peptides* 1995, 59, 131.
- [141] Heinrichs, S.C.; Lapsansky, J.; Lovenberg, T.W.; De Souza, E.B.; Chalmers, D.T. *Regulatory Peptides* 1997, 71(1), 15.
- [142] Mansbach, R.S.; Brooks, E.N.; Chen, Y.L. *Eur J Pharmacol* 1997, 323(1), 21.
- [143] Lundkvist, J.; Chai, Z.; Teheranian, R.; Hasanvan, H.; Barifai, T.; Jenck, F.; Widmer, U.; Moreau, J.-L. *Eur J Pharmacol* 1996, 309, 195.
- [144] Arai, K.; Ohata, H.; Shibasaki, T. *Neurosci Lett* 1998, 25(2), 103.
- [145] Iredale, P.; Alvaro, J.; Lee, Y.; Terwilliger, R.; Chen, Y.; Duman, R. *Soc Neurosci Abstr* 1999, 23, 529.
- [146] Timpl, P.; Spanagel, R.; Sillaber, I.; Kress, A.; Reul, J.M.; Stalla, G.K.; Blanquet, V.; Steckler, T.; Holsboer, F.; Wurst, W. *Nature Genetics* 1998, 19(2), 162.
- [147] Baram, T.Z.; Hatalski, C.G. *Trends in Neurosciences* 1998, 21(11), 471.
- [148] Webster, E.L.; Torpy, D.J.; Elenkov, I.J.; Chrousos, G.P. *Ann New York Acad Sci* 1998, 840, 21.
- [149] Chan, E.C.; Falconer, J.; Madsen, G.; Rice, K.C.; Webster, E.L.; Chrousos, G.P.; Smith, R. *Endocrinology* 1998, 139(7), 3357.
- [150] Smagin, G.N.; Howell, L.A.; Ryan, D.H.; De Souza, E.B.; Harris, R.B. *Neuroreport* 1998, 9(7), 1601.
- [151] Bornstein, S.R.; Webster, E.L.; Torpy, D.J.; Richman, S.J.; Mitsiades, N.; Igel, M.; Lewis, D.B.; Rice, K.C.; Joost, H.G.; Tokos, M.; Chrousos, G.P. *Endocrinology* 1998, 139(4), 1546.
- [152] Rodriguez de Fonseca, F.; Carrera, M.R.A.; Navarro, M.; Koob, G.F.; Weiss, F. *Science* 1997, 276, 2050.
- [153] Chen, Y.L. Pyrrolopyrimidines as CRF antagonists, 1994, WO 94/13676.
- [154] Chen, Y.L. Pyrazolopyrimidines as CRF antagonists, 1994, WO 94/13677.
- [155] Bright, G.M.; Welch, W.M.J. Substituted pyrazoles having corticotropin-releasing factor (CRF) antagonist activity, 1995, WO 95/33727.
- [156] Chen, Y.L. Pyrazolo and pyrrolopyrimidines, 1995, WO 95/34563.
- [157] Chen, Y.L. Pyrazolopyrimidines and pyrrolopyrimidines for treatment of neuronal and other disorders, 1996, EP 0 729 758 A2.
- [158] McCarthy, J.R.; Xie, Y.F.; Whitten, J.P.; Webb, T.R.; Chen, C.; Rainphal, J.Y. CRF receptor antagonists and methods relating thereto, 1998, US 5,795,905.
- [159] Volkmann, R.A. Benzimidazole derivatives and their use as corticotropin releasing factor antagonists, 1997, EP 0 812 831 A1.
- [160] Chen, Y.L. Substituted heterocyclic derivatives as CRF antagonists, 1997, EP 0 778 277 A1.
- [161] Webb, T.R.; Moran, T.J.; McCarthy, J.R. Amino substituted pyrimidines and triazines, 1997, WO 97/14684.
- [162] Bakthavatchalam, R.; Arvanitis, A.G.; Beck, J.P.; Cain, G.A.; Chorvat, R.J.; Gilligan, P.J. Arylamino fused pyridines and pyrimidines, 1997, WO 97/35539.
- [163] Chorvat, R.J.; Rajagopalan, P. Aryloxy- and arylthio-substituted pyrimidines and triazines and derivatives thereof, 1997, WO 97/35580.
- [164] Rajagopalan, P.; Chorvat, R.J.; Bakthavatchalam, R.; Beck, J.P.; Gilligan, P.J.; Olson, R.E. Aryloxy- and arylthio-fused pyridines and pyrimidines and derivatives, 1997, WO 97/35846.
- [165] Wilde, R.G. Tetrahydropteridines and pyridylpiperazines for treatment of neurological disorders, 1997, WO 97/44038.
- [166] Yuan, J. 3-aryl substituted pyrazolo(4,3-D) pyrimidine derivatives: corticotropin-releasing factor (CRF1) specific ligands, 1998, US 5,723,608.
- [167] Chen, Y.L. Substituted pyrido- or pyrimido-containing 6,6- or 6,7-bicyclic derivatives, 1998, WO 98/05661.
- [168] Rabinovich, A.K.; Dhanoa, D.S.; Luthin, D.; Bychowski, R.A.; Bhunalkar, D.R. Benzoperimidine-carboxylic acids and derivatives thereof, 1998, WO 98/08821.
- [169] Rabinovich, A.K.; Dhanoa, D.S.; Luthin, D.R.; Bychowski, R.A.; Bhunalkar, D.R. Benzoperimidine-carboxylic acids and derivatives thereof, 1999, US 5,861,398.

- [170] Chen, Y.L. Substituted 6,6-hetero-bicyclic derivatives, 1998, WO 98/08846.
- [171] Arvanitis, A.G.; Olson, R.E.; Arnold, C.R.; Fietze, W.E. Pyrazinones and triazinones and their derivatives thereof, 1998, WO 98/11075.
- [172] Fontaine, E.; Gully, D.; Roger, P.; Wernuth, C.G. Aminothiazole derivatives, method of preparation and pharmaceutical compositions containing same, 1998, WO 98/15543.
- [173] Yuan, J. Certain pyrazole derivatives as corticotropin-releasing factor receptor CRF1 specific ligands, 1998, WO 98/21200.
- [174] Yuan, J.; Yoon, T. Isoquinolinamine and phthalazinamine derivatives which interact with CRF receptors, 1998, WO 98/27066.
- [175] Tanaka, H.; Seio, K.; Kimura, K.; Minoguchi, M.; Uchida, M.; Kohara, T.; Ohashi, Y.; Morio, Y.; Yamagami, K. Fused pyrimidine compounds and medicinal use thereof, 1998, WO 98/29397.
- [176] Nakazato, A.; Kumagai, T.; Okubo, T.; Aibe, I.; Tanaka, H.; Chaki, S.; Okuyama, S.; Tomisawa, K. 4-Tetrahydropyridylpyrimidine derivatives, 1998, WO 98/42699.
- [177] Horvath, R.F.; Hutchinson, A. Certain pyrrolopyridine derivatives: novel CRF1 specific ligands, 1998, WO 98/45295.
- [178] Huang, C.; Wilcoxon, K.M.; Chen, C.; McCarthy, J.R. CRF antagonistic quino- and quinazolines, 1998, WO 98/47874.
- [179] Webb, T.R.; McCarthy, J.R. CRF antagonistic thiophenopyrimidines, 1998, WO 98/47903.
- [180] Cocuzza, A.J.; Hobbs, F.W.; Beck, J.P.; Gilligan, P.J. Aryl- and arylamino-substituted heterocycles as corticotropin releasing hormone antagonists, 1999, WO 99/01439.
- [181] Wilde, R.G.; Bakthavatchalam, R.; Beck, J.P.; Arvanitis, A.G. Imidazopyrimidines and imidazopyridines for the treatment of neurological disorders, 1999, WO 99/01454.
- [182] Adinolfi, B.; Anton, R.; Linnoila, M.; Guidotti, A.; Nemeroff, C.B.; Bissette, G. *Neuropsychopharmacology* 1996, 15(3), 288.
- [183] Baram, T.Z.; Chalmers, D.T.; Chen, C.; Koutsoukos, Y.; De Souza, E.B. *Brain Res* 1997, 770(1-2), 89.



PERGAMON

Journal of Psychiatric Research 33 (1999) 181–214

JOURNAL OF
PSYCHIATRIC
RESEARCH

The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety

F. Holsboer*

Max Planck Institute of Psychiatry, Kraepelinstr. 10, D-80804 Munich, Germany

Accepted 26 October 1998

Abstract

Neuroendocrine studies strongly suggest that dysregulation of the hypothalamic–pituitary–adrenocortical (HPA) system plays a causal role in the development and course of depression. Whereas the initial mechanism resulting in HPA hyperdrive remains to be elucidated, evidence has emerged that corticosteroid receptor function is impaired in many patients with depression and in many healthy individuals at increased genetic risk for an depressive disorder. Assuming such impaired receptor function, then central secretion of CRH would be enhanced in many brain areas, which would account for a variety of depressive symptoms. As shown in rats and also in transgenic mice with impaired glucocorticoid receptor function, antidepressants enhance the signaling through corticosteroid receptors. This mechanism of action can be amplified through blocking central mechanisms that drive the HPA system. Animal experiments using antisense oligodeoxynucleotides directed against the mRNA of both CRH receptor subtypes identified the CRH₁ receptor as the mediator of the anxiogenic effects of CRH. Studies in mouse mutants in which this receptor subtype had been deleted extended these findings as the animals were less anxious than wild-type mice when experimentally stressed. Thus, patients with clinical conditions that are causally related to HPA hyperactivity may profit from treatment with a CRH₁ receptor antagonist. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

For more than 100 years psychopharmacology has been shaped by compounds that have emerged from organic chemistry laboratories and whose systemic effects have then been studied by careful but unsystematic analysis. For example, in 1832 the German chemist Justus von Liebig synthesized chloral hydrate from ethanol and chlorinated lime. A few years later, in Boston, the American physician Charles T. Jackson accidentally observed the sedating effect of ether. This led researchers to test sedating and narcotic effects of other gaseous compounds such as chloroform. The German pharmacologist Oscar Liebreich postulated that chloroform might derive from chloral hydrate by degradation in blood, suggesting that chloral hydrate might be a sedative drug that could be administered orally. The first clinical trials, conducted in the department of psychiatry of the Charité Hospital in Berlin, confirmed the postulated sedative effect, although it turned out that it was not the decomposition of chloral hydrate into chloroform that caused sedation. Similar cases in which potential clinical applications for newly

developed chemical compounds were investigated led to the description of clinical indications for barbiturates and phenothiazines. A prominent serendipitous finding, made by the psychiatrist Roland Kuhn in Switzerland in the mid-50s, was for instance the discovery that heterocyclic compounds such as iminodibenzyl derivatives, to which imipramine belongs, have the property to act as antidepressants. All these developments have several characteristics in common: (1) The compounds were synthesized without any anticipation of their clinical utility; (2) their mode of action was unknown and the mechanism thought to be involved in the obvious clinical efficacy often turned out to be wrong; and (3) the clinical efficacy stimulated hypotheses about the causality of the respective disorder. For example, the Australian psychiatrist John Cade believed that lithium salts act through diathesis of uric acid, which produces psychosis. This hypothesis did not stand the test of time. Another pathogenetic hypothesis, departing from the pharmacology of antidepressants, postulated a central deficiency of bioavailable norepinephrine and serotonin. Thus, it was the antidepressants' mechanism of action itself that prompted the formulation of the biogenic amine deficiency hypothesis as put forward by Joe Schildkraut in Boston and Alec Coppen in London, and it may be said that no other hypothesis has influenced the development of anti-

* Tel.: +49 89 30622 220; fax: +49 89 30622 483; e-mail: holsboer@mpipsy.ki.inpg.de.

depressants to a similar extent. In this article, depression will serve as an example to illustrate that the classic 'from bench to bed' approach is now becoming more complex, as clinical and preclinical research identify central pathological mechanisms that can provide specific drug targets. One example of such a 'from bed to bench and back' strategy is the close interrelation between the dysregulation of the hypothalamic–pituitary–adrenocortical (HPA) activity in individuals with depression, the progression into depression, the action of current antidepressants, and the development of new drugs targeting HPA regulation.

2. Clinical evidence for CRH hyperactivity in depression

In response to acute physical or psychological stress, parvocellular neurons of the paraventricular hypothalamus (PVN) produce increased amounts of corticotropin-releasing hormone (CRH), which is released into portal vessels activating secretion of corticotropin (ACTH) from anterior pituitary cells. In turn, ACTH enters the circulation and elicits glucocorticoids from the adrenal gland. This rapid HPA activation can be life-sustaining because of the metabolic effect of elevating blood glucose levels. However, other stress-related responses needed for life-sustaining adaptations encompass a number of behavioral reflexes elicited by activation of the HPA system, presumably by an increase in CRH release (Fig. 1).

The many checks and balances of the HPA system serve to counter-regulate the stress-elicited activation. In individuals with major depression, however, there is sustained hyperactivity of the HPA system. In the following the evidence is reviewed that points to an unrestrained CRH hyperdrive in these patients, which would explain many of their symptoms.

Shortly after the group led by W. Vale at the Salk Institute in La Jolla, California, had isolated, sequenced and characterized CRH, synthetic ovine, rat and human neuropeptide probes became available for human studies (Vale et al., 1981). After a bolus of ovine CRH, patients with depression had ACTH responses that were indistinguishable from those of normal controls when the CRH was injected in the morning (Holsboer, 1983; Holsboer et al., 1984a) and attenuated when it was injected in the evening, when the HPA system is quiescent (Gold et al., 1984). Whereas the ACTH response to ovine CRH produces prolonged and higher peak concentrations than human CRH (hCRH), the latter more closely simulates the physiological condition (Orth, 1992). When hCRH is injected in the evening, it also produces attenuated ACTH responses in depressed patients (Holsboer et al., 1984b, 1986). These first studies and their numerous replications suggested that elevated

corticosteroid levels in combination with desensitized CRH receptors at corticotrophic cells might restrain the releasable amount of ACTH. Support for this view was provided by studies using metyrapone, which inhibits cortisol synthesis by blocking hydroxylation at the C11 position, showing that the ACTH blunting is avoided when plasma cortisol concentrations are lowered (von Bardeleben et al., 1988; Lisansky et al., 1989). A more recent study by Young et al. (1995) suggested that the evidence for CRH receptor desensitization at corticotrophic cells is mute as they found a 3-fold higher β -endorphin response to CRH in depressed patients pretreated with metyrapone than in those without pretreatment. Both β -endorphin and ACTH are synthesized and released from the pituitary after corticotrophic CRH receptor activation. In retrospect, the conclusions drawn from these studies are perhaps limited, as the enzyme block induced by metyrapone generates many other corticosteroid derivatives with neural activities of their own (Paul and Purdy, 1992; Rupprecht, 1997). For example, 11-deoxycorticosteroids are excessively increased after metyrapone, and studies by Patchev et al. (1994a, 1997) suggested that tetrahydrodeoxycorticosterone (THDOC), a major metabolite of 11-deoxycorticosterone, also interferes with CRH release and action.

Indirect evidence in favor of CRH receptor desensitization comes from studies with patients having panic attacks, in whom under non-panic conditions CRH-elicited ACTH is blunted despite normal plasma corticosterone levels at baseline. These studies were interpreted as indicative of CRH receptor desensitization, secondary to episodic increases of CRH during panic (Roy-Byrne et al., 1986; Holsboer et al., 1987a).

Although it seems difficult to determine whether blunted ACTH response is caused either by elevated corticosteroids or by desensitized CRH receptors, it is important to note that these are two closely related phenomena. To further explore these interdependencies, we pretreated both depressed patients and matched healthy controls with dexamethasone and observed that in the depressed patients the ACTH and cortisol responses were exaggerated whereas in the controls the ACTH and cortisol elevations were only mute (von Bardeleben and Holsboer, 1989, 1991). Age was found to be an important factor in enhancing ACTH and cortisol release (Heuser et al., 1996), and estradiol supplementation markedly attenuated the hormonal response to the dexamethasone-CRH test in postmenopausal women (Kudielka et al., unpublished observations). Systematic analysis of a broad data base revealed that this combined dexamethasone-(h)CRH test identified HPA dysregulation with high sensitivity in patients with affective disorders (Heuser et al., 1994). The explanation for the suppressing effect of basal cortisol on CRH-elicited ACTH versus the exaggerated ACTH response to low-dose dexamethasone pretreatment is based on the intriguing pharmacological

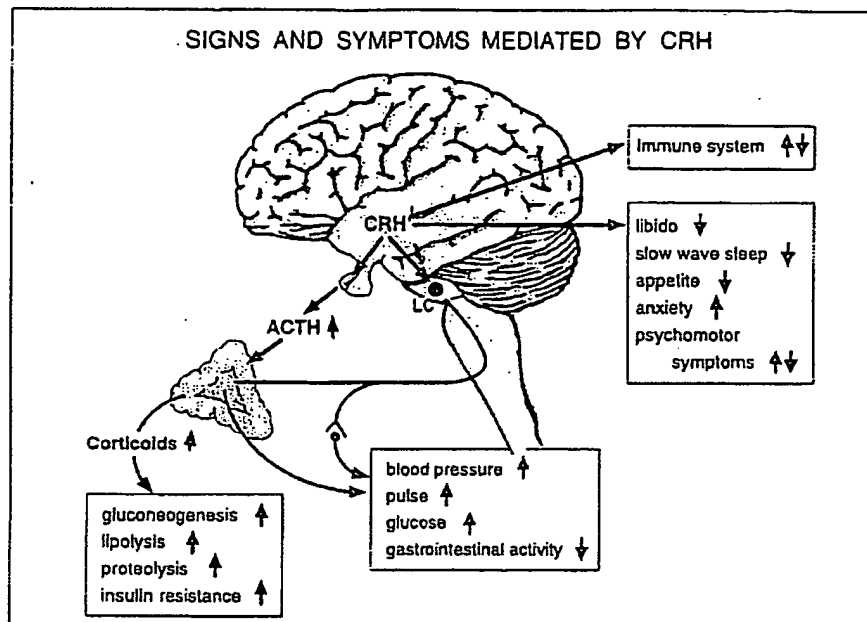


Fig. 1. Animal studies in which CRH was injected intracerebroventricularly or CRH synthesis was disrupted (knockout mice or antisense oligodeoxynucleotide treatment of rats) or CRH receptor function was manipulated through antagonists, gene therapy or gene targeting are in accord with indirect clinical evidence that many signs and symptoms present in depression can be attributed to enhanced secretory activity of central CRH.

differences between the two steroids: (1) Dexamethasone binds primarily to glucocorticoid receptors (GR), whereas cortisol also binds to GRs at corticotrophic and hypothalamic sites. In the hippocampus, however, which is also strongly involved in HPA regulation, cortisol, unlike dexamethasone, binds preferably to mineralocorticoid receptors (MRs) and only at high concentrations, for instance during stress, also to GRs; (2) in plasma, cortisol but not dexamethasone is bound to corticosteroid-binding globulin, which directs the action of dexamethasone to corticotrophic GRs; (3) the perhaps most important difference between cortisol and dexamethasone stems from the fact that dexamethasone is a substrate for the multidrug-resistant (mdr) 1a P-glycoprotein (Bourgeois et al., 1993), which is expressed in the apical membranes of endothelial cells of the blood-brain barrier (Cordon-Cardo et al., 1989). At low dosages, this mdr 1a P-glycoprotein acts as a pump, limiting passage of xenobiotic agents including dexamethasone from peripheral circulation into the brain (Schinkel et al., 1995). Meijer et al. (1998) have recently demonstrated that dexamethasone retention in the hippocampus and hypothalamus was much higher in mutant mice with a disrupted mdr1a gene, reaching levels in the order of pituitary retention, than in wild-type mice.

The consequence of the different mode of action of cortisol and dexamethasone is that in depressed patients

pretreated with a low dose of dexamethasone which, as already pointed out, acts primarily at the pituitary to suppress ACTH and, in turn, cortisol secretion, a deprivation of central natural ligands for GR and MR occurs. Because this loss is due to the limited access of dexamethasone to the brain, central regulatory sites sense this situation as adrenocortical insufficiency or a transient chemical adrenalectomy. In response, central HPA stimulants (ACTH secretagogues), mainly CRH and vasopressin (AVP), are hypersecreted and released via the median eminence into portal circulation. When CRH is injected under this condition, it synergizes with endogenously released AVP to override dexamethasone-induced suppression at corticotrophic cells. This interpretation was based on the finding that neither CRH nor AVP, when given alone, is capable of producing a major ACTH escape from dexamethasone suppression in controls (von Bardeleben et al., 1985; Wiedemann and Holsboer, 1997). However, when both neuropeptides were administered to dexamethasone-pretreated healthy controls, the resulting ACTH and cortisol levels were comparable to those of depressed patients, suggesting that among depressives not only CRH but also AVP is hypersecreted (von Bardeleben et al., 1985; von Bardeleben and Holsboer, 1989). Further evidence in support of this hypothesis was recently provided by the group of Dick Swaab in Amsterdam, who found increased num-

bers of both CRH-secreting neurons and CRH neurons that coexpressed AVP mRNA in the hypothalami of depressed patients (Raadsheer et al., 1994; Purba et al., 1996) (Fig. 2). In line with this is the report by Nemeroff et al. (1988) of a decrease in the number of CRH binding sites in the frontal cortex of depressed patients who committed suicide. This has been interpreted as an adaptive (homologous) down-regulation in response to increased CRH secretion. Two groups in England were unable to reproduce these findings, however, and could not confirm changes in CRH immunoreactivity or CRH binding sites in the cortices of depressed suicides (Charlton et al., 1988; Hucks et al., 1997; Leake et al., 1990), which probably reflects methodological difficulties that are not unlikely to be encountered in such studies. For example, the impact of the different violent methods used for committing suicide or the type of antidepressant treatment may be among the more important confounds.

Another line of evidence that CRH is not only a determinant of neuroendocrine signs of depression but is also causally involved in depressive psychopathology emerged from the work of Charles Nemeroff and colleagues in Atlanta, U.S.A. This group conducted a series of studies in which the CRH concentration in the cerebrospinal fluid (CSF) was measured by radioimmunoassay in drug-free depressed patients, patients in other diagnostic categories, and controls (Nemeroff et al., 1984; Banki et al., 1987; Arato et al., 1989). These and numerous other studies confirmed that CRH is elevated in the CSF of

patients with severe depressive illness, and importantly, that after successful drug treatment CSF levels of CRH had decreased again (De Bellis et al., 1993). Several other studies could not substantiate these CRH elevations in the CSF of depressives, however (Molchan et al., 1993; Pitts et al., 1995; Geraciotti et al., 1997). In this context, several factors need to be considered. The group led by Nemeroff used patients who were more acutely ill, and their studies and those of others indicate that the likelihood of CRH elevations increases with the presence of peripheral signs of HPA overactivity. In contrast, patients with eucortisolemic depression as studied by Geraciotti et al. (1992, 1997) do not seem to have elevated CRH concentrations in the CSF, which calls for studies investigating the extent to which peripheral cortisol concentrations may influence the activity of CRH neurons through actions on central CRH neurons. In rats, Swanson and Simmons (1989) showed that corticosteroids may activate CRH expression in the dorsal-parvocellular part of the hypothalamic PVN. From there, hypothalamic spinal projections of CRH neurons emerge, which suggests that CRH in the CSF is possibly a reflection of HPA activity, with cortisol acting as a stimulus for spinal CRH.

Taking all these clinical studies together, it seems fair to say that the CRH hypothesis is well founded, although several questions remain open. Of course, the limits of clinical examinations call for preclinical studies in animal models and basic studies at the cellular and molecular level to better understand how CRH is regulated and

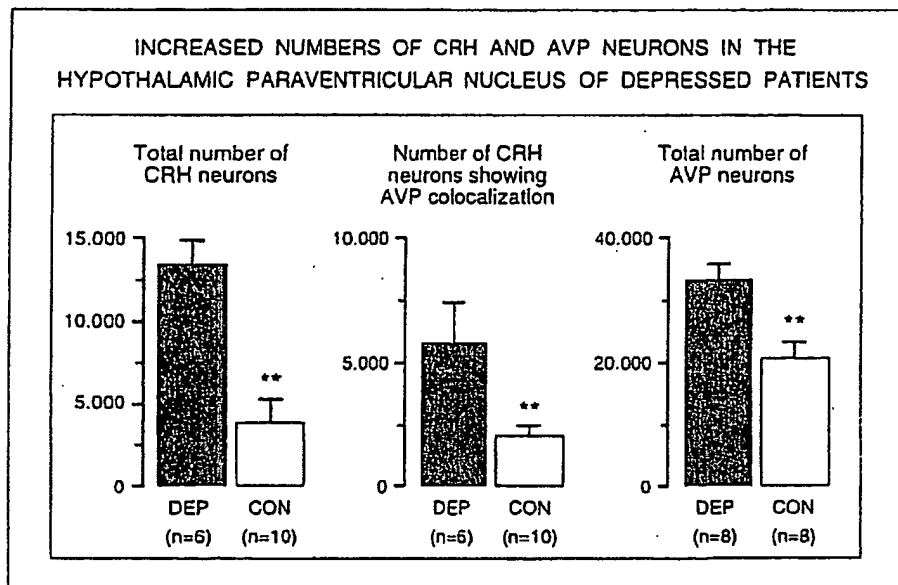


Fig. 2. Patients with major depression have an increased number of CRH and arginine vasopressin (AVP) neurons and also increased co-packaging of CRH and AVP in PVN neurons, which explains the excessive HPA activity in these patients as both neuropeptides synergize their actions at pituitary CRH receptors. DEP = depressed patients; CON = controls (adapted from Raadsheer et al., 1994 and Purba et al., 1996).

which regulatory elements might serve as potential drug targets of the HPA system.

3. Behavioral effects of CRH in animals

Only a few other neuropeptides have been studied more extensively than CRH with regard to their behavioral effects. Several reviews covering this issue have been published (Koob and Bloom, 1985; Dunn and Berridge, 1990; Holsboer et al., 1992; Owens and Nemeroff, 1992), which is why only a brief summary will be given of the main evidence that CRH acts as a mediator of affective symptoms.

Neuroanatomical studies strongly suggest that CRH not only accounts for neuroendocrine adaptations to stress, but that it may also have numerous other effects as CRH neurons have a widespread but selective distribution throughout the central nervous system (CNS). As shown by Swanson and Simmons (1989) and Merchenthaler (1994), the hypothalamic PVN is the major site of CRH-containing cell bodies. These cell bodies send axon terminals to the capillaries of the median eminence, from where CRH enters portal circulation to regulate proopiomelanocortin (POMC)-derived peptide (mainly ACTH and β -endorphin) synthesis and secretion from pituitary corticotrophs. Other CRH neurons of PVN origin project to the brainstem and spinal cord, both of which contain CRH cell bodies and influence behavioral activity and autonomic function. Spinal cord CRH neurons may also modulate sensory input through ascending pathways and, additionally, may represent preganglionic neurons that modulate sympathetic outflow.

The central nucleus of the amygdala (CeA) and the bed nucleus of the stria terminalis (BNST) contain large and discrete populations of CRH perikarya. Whereas CRH neurons from the BNST project to the brainstem, CRH neurons in the CeA send terminals to parvocellular regions of the PVN. These morphological findings together with the findings that CRH fibers interconnect the CeA, PVN and BNST clearly indicate that autonomic and neuroendocrine actions of CRH are functionally intertwined. A CRH involvement in complex behaviors is suggested by the presence of CRH interneurons, localized to layers II and III of the cortex. Although CRH-containing neurons are present throughout the neocortex, particularly high densities are found in the prefrontal cortex, emphasizing a role of CRH in cognitive processes.

Most studies exploring behavioral effects of CRH in animals have used intracerebroventricular (icv) or site-specific injections of CRH, and all agree that CRH mediates numerous anxiogenic and fear-related aspects of stress. When injected into rats or mice in a novel environment, CRH increases grooming and freezing behavior and decreases the number of approaches to a food pellet (Britton et al., 1982; Sutton et al., 1982).

In the conflict test, CRH suppresses both punished and nonpunished responding, which is another indication of anxiety-related behavior (Britton et al., 1986). Other studies in rats demonstrated a CRH-induced potentiation of acoustic startle (Swerdlow et al., 1989), a suppression of social interaction (Dunn and File, 1987), and an increase in stress-induced freezing behavior (Sherman and Kalin, 1988). Furthermore, symptoms of behavioral despair were observed in investigations of adult rhesus monkeys (Kalin, 1990). Most of these behavioral effects were blocked by α -helical CRH_{41–49}, which acts as a CRH receptor antagonist, but has intrinsic effects by itself (see below). Administration of CRH to neurons of the locus coeruleus (LC) has excitatory effects (Valentino et al., 1983), which prompted Butler et al. (1990) to study the behavioral effects of CRH microinfusion into the LC. These authors used a modified version of the open field test to monitor anxiogenic effects and showed that with increasing CRH dosages the exploratory behavior decreased, while the time spent in the darkened compartment increased. This dose-dependent surge in anxiety-related behavior was accompanied by increased norepinephrine turnover in forebrain areas, to which noradrenergic neurons project.

All these experiments were built upon increases in anxiety-related behavior as elicited by high CRH dosages in normal rats. More recently, the possibility of studying the effects of decreased CRH neurotransmission by administering antisense oligodeoxynucleotides (ODNs) corresponding to the start-coding region of CRH mRNA was employed. With this technique, translation of CRH mRNA into CRH is suppressed, enabling assessment of whether the decreased CRH concentrations lead to a suppression of anxiety under basal and stress conditions. Skutella et al. (1994a,b) used the shuttle-box experiment and the social defeat paradigm to measure the anxiety-related behavior in rats by assessing the entries into and time spent on the open arms of the elevated plus-maze. As expected, anxiety-related behavior was decreased by CRH antisense ODN probes and the specificity of this treatment with regard to CRH targeting was confirmed by decreases in CRH concentrations in the PVN and reduced ACTH plasma levels. A different approach was used by Stenzel-Poore et al. (1994), who bred transgenic mice overexpressing CRH. These mice have deficits in emotionality and can serve as genetic models of anxiogenic behavior. In addition to increased anxiety-related behavior, which is reversible after administration of a CRH receptor antagonist, CRH transgenic mice also show memory impairments (Heinrichs et al., 1996). Moreover, CRH is also capable of inducing a number of other behavioral changes that can be extrapolated to human affective disorders. Among these are CRH-elicited changes in sleep as measured by electroencephalography. If CRH is administered centrally to rats or peripherally to healthy controls, slow wave sleep,

the part of sleep that is believed to have recuperating effects, is decreased and sleep onset-related growth hormone release is blunted (Ehlers et al., 1986; Holsboer et al., 1988). Both phenomena are also common in individuals with depression (Steiger and Holsboer, 1997).

Psychomotor changes are also frequent in these patients, and CRH can increase locomotor activity as demonstrated by studies in rats (Sutton et al., 1982). Decreased sexual drive is another cardinal symptom of depression, and therefore it is important that reproductive behavior is potently inhibited by central administration of CRH (Sirinathsinghji, 1986). Furthermore, transgenic mice overexpressing CRH exhibit reproductive deficits due to decreased hypothalamic-pituitary-gonadal activity, which can be interpreted as being responsible for decreased sexual interest.

Most patients with major depression have a loss of appetite. Consequently, weight loss is a frequent symptom in these patients, and chronically stressed people often become anorectic. Chronic icv injections of CRH into rats produces weight loss because of decreased food intake, suggesting that a CRH excess is also involved in stress-associated anorexia. However, these and many other studies have used high dosages of CRH, and there is no doubt that anorexia, sleep disorders, anxiety, locomotor activity and sexual behavior are regulated in much more complex ways, with a large number of different neuropeptides and their respective receptors being involved. It is also of note that the effects observed after manipulation of central CRH levels are consistent with stress-related behavioral adaptations. In this context, anxiety is certainly the best-documented phenomenon so far. Because it remains unprovable whether depression exists in rodents, it also remains open whether CRH produces depression in these animals. However, the examples selected provide compelling evidence that many cardinal symptoms characteristic of depression are most likely mediated by CRH.

4. Involvement of CRH in central neurotransmitter systems

The high density of CRH-immunoreactive fibers in the LC, which contains almost 50% of the brain norepinephrine (NE) neurons, and the recent evidence for synaptic contacts between CRH terminals and LC dendrites (van Bockstaele et al., 1996) have led to many studies exploring how hormonal, autonomic and behavioral effects of stress are co-ordinated through interactions of CRH with the LC (Valentino et al., 1993). As mentioned earlier, infusion of CRH into the LC of freely moving rats produces an increase in catecholamine activity and turnover in the frontal cortex along with increased anxiety-related behavior (Butler et al., 1990).

This is in keeping with experiments by Valentino and Webby (1988), which show that CRH administration, like acute stress, increases the firing rate of LC neurons, whereas administration of a CRH antagonist blocks this effect. Chronic stress also increases the expression of tyrosine hydroxylase, the rate-limiting enzyme of catecholamine biosynthesis. That stress-elicited CRH participates in this action as well can be demonstrated by treatment with the α -helical CRH₄₁₋₄₄ antagonist which effectively prevents the induction of this enzyme (Melia and Duman, 1991).

Other brain regions that are both innervated by CRH terminals (Swanson and Simmons, 1989) and also contain mRNA for CRH receptors (Chalmers et al., 1995) are the dorsal and median raphe nuclei, from where serotonergic projections to the forebrain emerge. Stress is believed to activate 5-hydroxytryptamine (5-HT) from raphe nuclei, and because CRH innervates this brain region, studies have been conducted to investigate how stress-elicited CRH and 5-HT release are linked. Singh et al. (1991) found that CRH increased the activity of tryptophan hydroxylase, the rate-limiting enzyme in the synthesis of 5-HT, in midbrain and cortex. A more recent study by Price et al. (1998) demonstrated that icv administration of CRH to freely moving rats has biphasic effects as the dosages of 0.1 and 0.3 μg decreased the dialysate 5-HT concentrations in the lateral striatum while 3.0 μg increased them. Because forebrain 5-HT levels are strongly affected by behavioral arousal (Rueter and Jacobs, 1996), the increase in 5-HT after very high CRH dosages may be related to behavioral activation. Linthorst et al. (1997) monitored 5-HT release by microdialysis in the hippocampus of freely moving rats and also assessed the behavioral activation by visual observation. After infusion of a low dose of CRH ($1 \mu\text{g} \cdot \mu\text{l}^{-1} \cdot \text{h}^{-1}$) for 7 days into the brain of these rats, hippocampal 5-HT levels remained unchanged. However, if CRH-treated rats were stressed by intraperitoneal injections of lipopolysaccharide, they exhibited a blunted 5-HT response (Fig. 3) and delayed onset of behavioral inhibition.

These experiments suggest that stress-elicited CRH, by acting on dorsal raphe nuclei, is capable of inhibiting 5-HT activation.

A fairly close relationship has been suggested to exist between stress-elicited CRH and γ -aminobutyric acid (GABA)-gated chloride channels. Imaki and Vale (1993) showed that benzodiazepines are able to suppress the synthesis and release of CRH. The suppressive effect of benzodiazepines on HPA activation is clinically well documented and is supported by neuroanatomical studies, which show that dense GABAergic innervation is present in hypothalamic nuclei that control anterior pituitary function. The GABA/benzodiazepine sites are also present in hippocampal and amygdala regions that are under CRH control and provide a route for neocortical effects on the hypothalamus (Swanson et al., 1983; Rich-

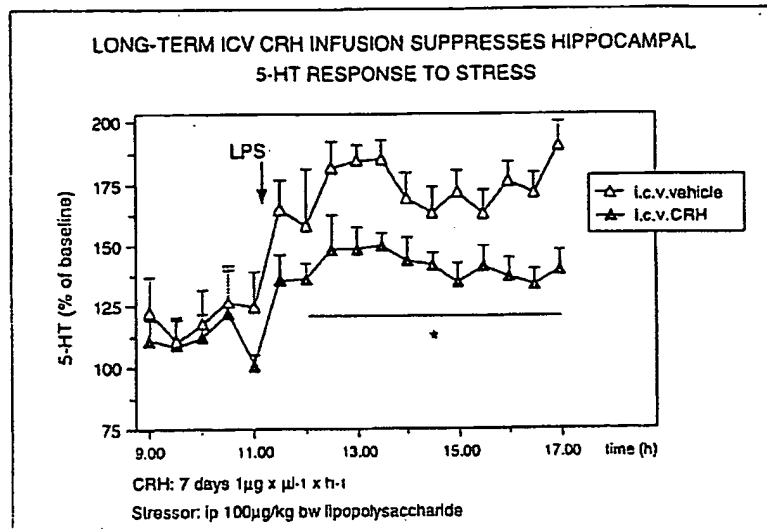


Fig. 3. Rats were intracerebroventricularly (icv) infused with either CRH ($1 \mu\text{g}/\mu\text{l} \times \text{h}$) or vehicle. When both groups were stressed with lipopolysaccharide ($100 \mu\text{g}/\text{kg}$ intraperitoneally), those rats treated with CRH responded with a comparatively minor serotonin release in the hippocampus, as measured by in vivo microdialysis (from: Linthorst et al., 1997).

ards et al., 1987; Sakaue et al., 1988). These CRH effects appear to be negatively controlled by GABAergic actions. The involvement of GABA-gated ion channels is also supported indirectly by studies showing that neuroactive steroids such as allopregnanolone (tetrahydropregesterone) and THDOC are capable of suppressing CRH, which would explain their anxiolytic effects (Patchev et al., 1994b, 1997). The effect of benzodiazepines on HPA activity has also been studied by Owens et al. (1989), who showed that administration of alprazolam decreased the CRH content in the LC. More recently, Wilson et al. (1996) demonstrated that these effects are influenced by gender, which may also explain the sexual dimorphism in stress-induced corticosterone and ACTH release.

Excitatory amino acids (EAAs) have a stimulatory effect on ACTH and cortisol secretion (Jezova and Oprslova, 1992), but it is not quite clear whether EAAs act at a central level to co-regulate the synthesis and release of ACTH through specific binding sites (Meeker et al., 1994). The possibility that EAAs activate the HPA system via CRH is supported by the finding that *N*-methyl-D-aspartate (NMDA)-increased cortisol release is prevented by icv infusion of antiserum against CRH in rhesus monkeys (Reyes et al., 1990). Moreover, in vitro studies analysing the secretion of CRH from hypothalamic slices showed that EAAs, through NMDA and metabotropic receptors, but not through kainic or AMPA receptors, activate CRH secretion (Joanny et al., 1997). In contrast, other studies found no CRH release (Costa et al., 1992) or decreased CRH release (Patchev

et al., 1994a) after exposure to EAAs, which was related to methodological differences. Nevertheless, in vivo studies support a CRH-activating effect of EAAs either through direct hypothalamic actions or through afferents subserving CRH cell bodies because systemic administration of MK801 (a non-competitive antagonist of NMDA receptors) suppresses stress-induced HPA activation. The interaction between CRH and EAAs is of particular interest since EAAs, acting mainly via NMDA receptors, produce neurotoxic effects that are believed to be a primary cause of focal ischemic brain damage. Moreover, CRH is suspected of mediating neuronal damage induced by focal ischemia or NMDA receptor activation, perhaps through direct effects on neuronal activity (Aldenhoff et al., 1983; Strijbos et al., 1994). In line with this suggestion is the finding that glutamate- or ischemia-induced infarction size is decreased by co-administration of an α -helical CRH receptor antagonist. Likewise, ethanol withdrawal, which is accompanied by increased CRH release and anxiety-related behavior, is also accompanied by increased concentrations of CSF indices of EAA neurotransmission (Tsai et al., 1998), which may lead to oxidative stress and subsequent neurodegeneration (Coyle and Puttfarcken, 1993; Behl, 1998).

5. Causation of increased CRH in depression

Given that excessive CRH accounts for the well-documented HPA hyperdrive and a number of autonomic

signs and psychopathological symptoms in individuals with depression, the question of why CRH is not adequately regulated in these patients remains. The best-studied brain regions are the hippocampus and the hypothalamic PVN, where adrenalectomy was repeatedly shown to stimulate CRH biosynthesis and release to an extent similar to that seen in profound stress (Antoni, 1986; Plotsky, 1990; Dallman, 1993). Activation of CRH and subsequently of ACTH and corticosterone can also be achieved by GR antagonists and glucocorticoid synthesis inhibitors. Over time, there is increasing participation of AVP in HPA activation. This neuropeptide can also induce anxiety-related behavior (Landgraf et al., 1995). As already mentioned, similar to the human condition in depression, chronic stress in rats is associated with increased CRH and AVP co-expression and co-release from the PVN via the median eminence into the portal vessels (de Goeij et al., 1991). The effect of adrenalectomy is counteracted by exogenous corticosterone, and at low dosages only AVP synthesis is affected, indicating that AVP expression is more sensitive to corticosteroid feedback signals than CRH expression (Bradbury et al., 1994). Within the limits of neuroendocrine HPA regulation it seems clear that corticosteroids restrain CRH and AVP expression through GR activation. Because glucocorticoids are elevated in most patients with major depression, the question arises of whether there is a GR resistance in depression and if so whether this resistance is inherited or acquired.

Modell et al. (1997) performed the combined dexamethasone/CRH test in patients and controls, using increasing dosages of dexamethasone before stimulation with a fixed CRH dose. As illustrated in Fig. 4, depressed patients showed a shift of the response curve toward lower sensitivity. This effect was much less pronounced after clinical remission. Healthy subjects at genetic risk for depression also show this phenomenon as a trait (Holsboer et al., 1995b; Modell et al., 1998), suggesting the presence of GR resistance in a population with inherited susceptibility for depression. In such individuals, glucocorticoid signaling may be disturbed to a degree that does not precipitate depression under normal conditions. However, under chronic stress the impaired GR signaling may ultimately lead to cellular dysregulation that can no longer be compensated, resulting in the clinical syndrome. Work by Kendler (1998) in New Haven, U.S.A., suggests that the depressogenic effect of stressful life events is substantially greater in individuals at genetic risk for depression. Future results from the Munich Vulnerability Study will show whether those individuals who are at risk because of a high genetic load for depression and who show aberrant HPA symptoms will exhibit a manifestation of depression in later life (Lauer et al., 1998). Alternatively, the impaired GR signaling in individuals at genetic risk may render them susceptible for stressful life events. This possibility is also

in keeping with a study by Kendler and Karkowski-Shuman (1997) suggesting that genetic factors influence the kinds of stressful life events to which people expose themselves. Those with inherited premorbid HPA dysregulation due to decreased GR signaling are perhaps the ones who expose themselves to harmful life events, resulting in insufficiently restrained HPA activation, which subsequently results in depression through excessive CRH synthesis and release.

It is important to recognize that CRH can activate its own expression in the PVN. Parkes et al. (1993) injected CRH into the lateral ventricle of rats and showed that two separate immediate early genes, *c-fos* and *nur77* (also called NGFI-B), were expressed, followed by increased expression of CRH mRNA as quantified by in situ hybridization. This mechanism is perhaps life-sustaining as it keeps the organism responsive to acute stressors under conditions of chronic stress. Under such conditions, negative feedback through corticosteroids is weakened. Two examples using mouse mutants demonstrate that there is no simple reciprocal interaction between GR function and CRH expression. A transgenic mouse expressing antisense directed to GR mRNA was generated by inverting a 1.815-bp fragment of the 3'-noncoding region of GR cDNA downstream from a 2.3-kb *EcoRI/Hind III* human neurofilament promoter and inserting it into the mouse genome (Pepin et al., 1992). These mice had an exaggerated ACTH response to stress corresponding to the deficit in GR-mediated repression of POMC gene transcription. To achieve this transrepression, corticosterone must bind to intracellular GRs, which after dissociation from chaperone proteins (heat shock proteins) dimerize and bind to negative glucocorticoid response elements (GRE) in the POMC promoter. The transgene expression in these mice apparently interferes with this mechanism, resulting in an excessive ACTH response to stress (Barden et al., 1997). In contrast to the POMC gene, from which ACTH derives, expression of the CRH gene is not increased but rather is reduced in the PVN and in the external zone of the median eminence (Dijkstra et al., 1998). To understand this, it is important to know that unlike POMC expression transrepression of CRH does not involve DNA binding of ligand-activated and dimerized GRs at GREs. The suppressive effect of GRs on CRH gene expression is achieved through the interaction of activated GRs with other proteins distant from DNA-binding sites. Thus, GRs can suppress transactivation by binding to other transcription factors. Such protein-protein interactions may include binding between an activated GR and the cAMP response element-binding protein (CREB), which activates CRH gene expression in the CRH gene promoter through cAMP response elements (CRE) (Spengler et al., 1992).

Another interesting possibility is a hypothesized interaction with the orphan receptor *nur77*, which acts as a

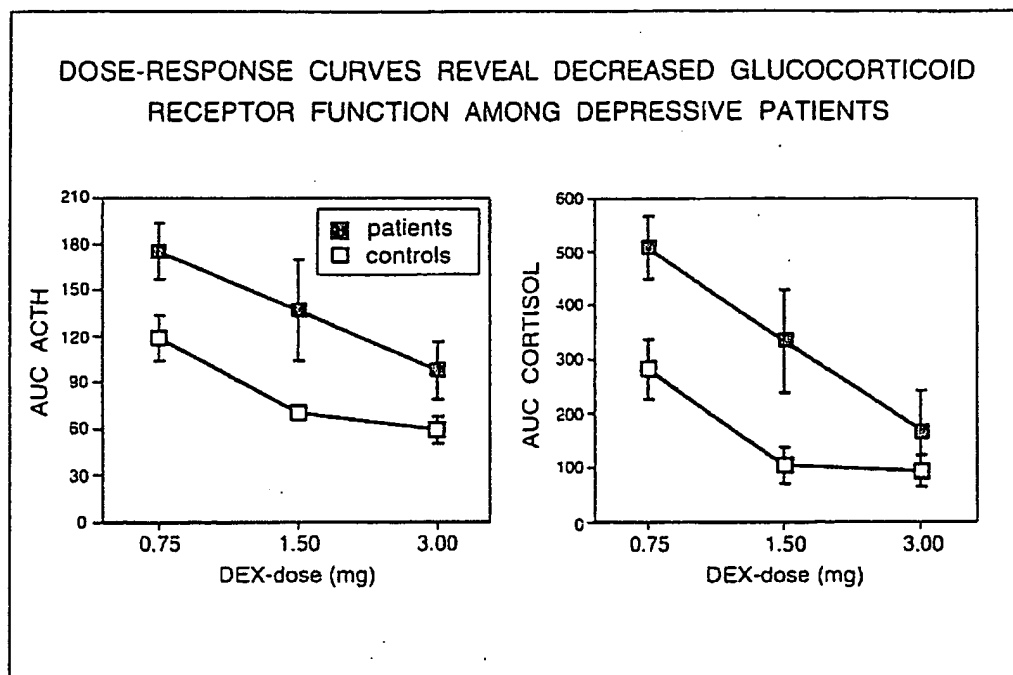


Fig. 4. Among 30 patients with major depression three different dosages of dexamethasone (DEX) (given orally at 2300 h) had a less suppressive effect on the releasable amount of ACTH and cortisol (both expressed as area under the time course curve, AUC) than among the group of matched controls, which indicates an impaired corticosteroid receptor function among depressed patients (from: Modell et al., 1997).

nuclear transcription factor that binds as an unliganded monomer to a specific DNA motif, activating expression of the CRH gene for which a *nur77* response element has been identified in the CRH gene promoter (Murphy and Conneely, 1997). Sequence analysis of the cDNA identified *nur77* as a member of the steroid receptor superfamily, and it is not unlikely that the GR heterodimerizes with *nur77* to suppress CRH gene expression because binding of *nur77* to the GR prevents CRH gene activation through the *nur77* DNA response element.

With regard to the enhanced ACTH secretion but suppressed CRH secretion in transgenic mice with impaired GR function, it appears that the genetic manipulation has preserved the GR capacity to suppress CRH gene expression through protein-protein interaction. In contrast, the transrepression requiring DNA binding of activated GR homodimers at negative GREs in the POMC promoter is apparently defunct in these mutants, resulting in enhanced ACTH release under stress. A similar dualism of HPA regulation through GR was recently observed by Reichardt et al. (1998). These authors used the finding by Heck et al. (1994) that introduction of a point mutation (arginine 458 threonine) into the amino acid sequence of the second zinc finger in the DNA-

binding domain of the GR prevents transactivation of GRE-dependent promoters. By gene targeting using the *Cre/loxP* system, Reichardt et al. (1998) created a mouse mutant in which it was possible to differentiate between transrepression and transactivation of the HPA system. Because of the mutation, these mice had elevated POMC mRNA and ACTH levels in the anterior pituitary, which reflects the need for DNA binding of GRs to exert negative feedback at the pituitary level in vivo. However, the CRH content in the median eminence was unaffected, suggesting that GR-GR dimerization is not necessary for transrepression through interaction with other transcription factors at the hypothalamic level. What still needs to be investigated is the mode of regulation of CRH in other brain structures such as the amygdala, which modulates emotional responses to stress (Gallagher and Chiba, 1996) and which has also been suggested to be implicated in conditioned fear in humans (LaBar et al., 1998). In the context of inherited impairment of corticosteroid signaling in depression, these observations are instructive insofar as they indicate that regulation of gene expression through corticosteroid receptors involves a complex nuclear assembly of transactivating factors, minor changes of which can contribute to pathology.

These transactivating factors may also serve as potential drug targets.

Whereas the studies referred to earlier focus mainly on the question of inherited dysfunction of the HPA system as a risk factor for depression, a number of animal studies have demonstrated that pre- or postnatal stressors may also affect the HPA system lifelong. Reul et al. (1994a) showed that prenatal immunostimulation of pregnant rats leads to persistent HPA hyperactivity in offspring. Other studies also showed that prenatally stressed rats have increased amygdala CRH concentrations later on and display an exaggerated hippocampal acetylcholine responsiveness following administration of CRH (Day et al., 1998).

In a series of elegant experiments, the groups of Charles Nemeroff and Paul Plotsky in Atlanta, U.S.A., studied the effects of maternal care on infant rats. They showed that those rats that had received frequent maternal care (licking, grooming) during the first 10 days of their life displayed reduced plasma ACTH and corticosteroid concentrations after stress, increased hippocampal GR mRNA and feedback sensitivity, and decreased hypothalamic CRH mRNA when they were grown-up (Liu et al., 1997). In a subsequent report the authors showed that adult rats whose mothers had licked and groomed them frequently in early infancy also had increased benzodiazepine receptor density in the amygdala and LC, increased α_2 -adrenoceptor density in the LC and decreased CRH receptor binding in the LC (Caldji et al., 1998). In contrast, rats that were postnatally traumatized by maternal deprivation alone or in combination with mild foot shocks had an increased CRH concentration in the median eminence and a decreased number of corticotropin CRH receptors (Ladd et al., 1996).

In addition to these experiments in rats, Nemeroff's group also studied primates that were exposed to adverse rearing conditions in infancy. When grown-up, those monkeys that were raised by their mothers under unpredictable foraging conditions had persistently higher CSF concentrations of CRH than those animals whose mothers had regular access to food (Fig. 5) (Coplan et al., 1996). These findings support the view that vulnerability to depression can also be acquired through early trauma such as neglect or childhood abuse. However, these important findings do not allow the conclusion that maternal deprivation per se results in long-term effects on cognition or HPA function in all affected individuals. The group led by Ronald de Kloet in Leiden, the Netherlands, studied Brown Norway rats, known for their long and healthy life span. When litters were postnatally removed from their mothers for 24 h, about half of the animals had very good cognitive abilities as adults (when aged 30 months) compared to the others who performed poorly. In those who were reared by their mothers without interruption, good and poor learning performance was normally distributed, showing that sus-

ceptibility to early trauma is influenced by individual genetic predisposition (de Kloet et al., 1998).

6. Suppressing the HPA hyperdrive with antidepressants

Serial monitoring of HPA activity and severity of depressive symptoms during treatment with antidepressants revealed that excessive HPA activity gradually decreases and that this effect precedes full clinical recovery, which suggests that normalization of stress hormone regulation is a prerequisite for clinical recovery (Holsboer, 1995a). Such a causal link between neuroendocrine signs and psychopathological symptoms is further supported by two recent observations: (1) patients who do not respond to antidepressant treatment continue to have HPA dysregulation (Holsboer et al., 1987b; Holsboer-Trachsler et al., 1994; Heuser et al., 1996) and (2) patients who are fully remitted but still have HPA dysregulation as measured with the combined dexamethasone-CRH test have a much higher risk for relapse within 6 months than patients who are fully remitted with regard to both psychopathology and neuroendocrine signs (Zobel et al., 1999).

These and many similar findings have led us to modify the description of the course of depressive illness as proposed by Kupfer (1993) in a way that takes the observed HPA dysfunction into account (Fig. 6). As the time course patterns do not appear to be influenced by the type of antidepressant, a number of studies were performed to challenge the hypothesis that antidepressants act by normalizing the HPA system. Antidepressant actions include an increase in the efficiency of glucocorticoid signaling and thus enable a more potent suppression of hormone-regulated genes either through GR interactions with other transcription factors (protein-protein interactions) as described earlier or through GR binding at DNA sequences other than the well-characterized consensus glucocorticoid response element (GRE) sequences (Phi Van et al., 1990; Malkoski et al., 1997).

The possibility that antidepressants can act by increasing GR signaling efficiency, regardless of their specific pharmacology, was suggested by Pepin et al. (1989), who showed that various antidepressants can increase GR mRNA in primary cultures of rat brain hypothalamic neurons and the amygdaloid complex. Interestingly, the tricyclic antidepressant desimipramine, which is primarily a noradrenaline reuptake inhibitor, induces activation of the GR promoter in fibroblast (LTK⁺) cells. These cells do not secrete catecholamines, which suggests that the mechanism by which tricyclics influence GR activity does not necessarily involve noradrenaline reuptake. The aforementioned mouse mutant expressing a transgene that results in functional GR impairment showed a normalized ACTH response to stress and

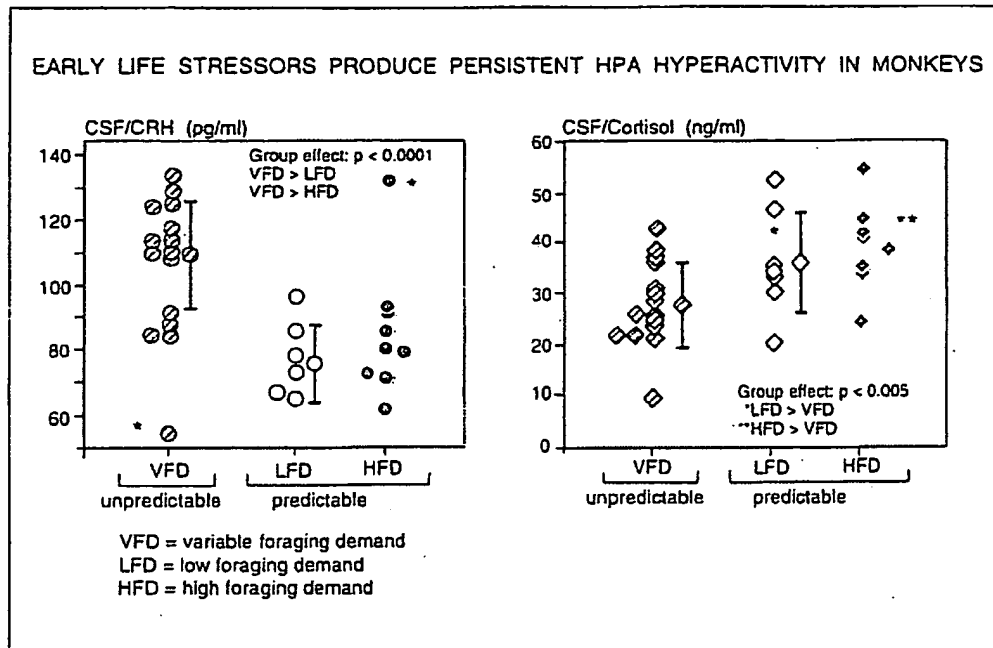


Fig. 5. Thirty bonnet macaques (*Macaca radiata*) were reared under different conditions. The HFD mothers were required to dig through wood-chip to obtain their daily food ration. During the 12-week rearing period the LFD mothers were offered abundant food items that could be picked up from the floor. The VFD mothers experienced varying conditions, either two weeks of HFD conditions or two weeks of LFD conditions. CSF samples taken a few years later from the grown-up offspring revealed persistent changes in CRH secretion, with increased levels among those grown primates that had been reared under unpredictable (VFD) foraging conditions (adapted from: Coplan et al., 1996).

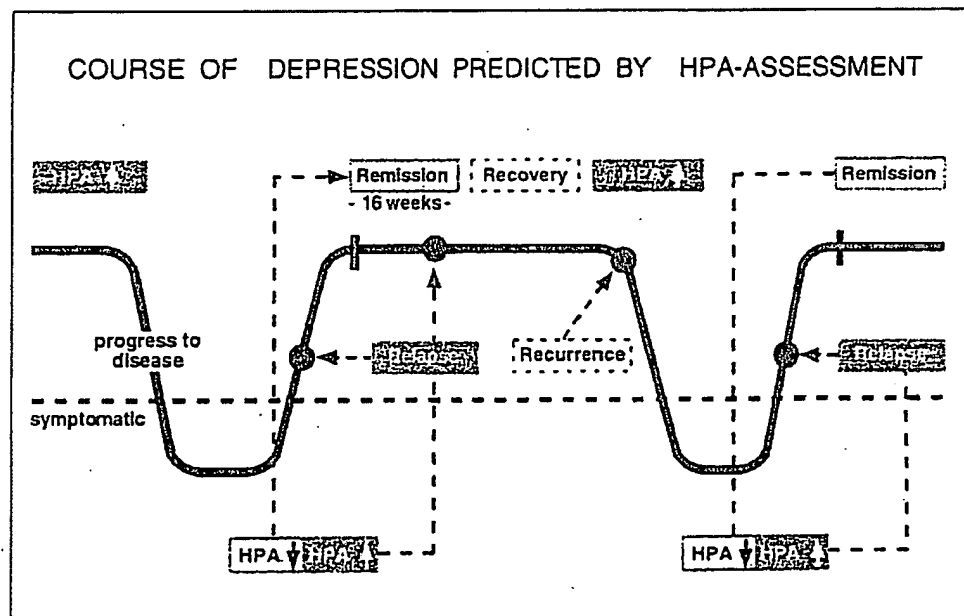


Fig. 6. Postulated relationship between the course of depression and HPA regulation.

improved cognitive performance after administration of the antidepressant moclobemide, a reversible inhibitor of monoamine A. (Montkowski et al., 1993), and an increased hippocampal (CA1 region) long-term potentiation (Steckler et al., unpublished observations; Fig. 7), an electrophysiological measure of neuroplasticity. Changes in long-term potentiation are also frequently accompanied by alterations in memory and learning, as shown by Bliss and Collingridge (1993). Reul et al. (1993, 1994b) studied HPA changes in rats after long-term treatment with amitriptyline (a pharmacologically unspecific tricyclic compound) and moclobemide. They administered antidepressants in time and dose regimens that are equivalent to clinical applications. The first change to be observed after two weeks was an increased MR binding, followed by increased GR binding after five weeks in the hippocampus and other brain regions, e.g., the hypothalamus, amygdala and neocortex. After antidepressant treatment for five weeks the rats showed a decreased HPA response to stress. Other studies also reported that impaired negative feedback in rats (Rowe et al., 1997) and in humans (Michelson et al., 1997) can be restored by antidepressants (Holsboer and Barden, 1996).

Intrigued by the hypothesis that increased efficiency of MR signaling is a first important step in antidepressant action (Reul et al., 1993), a clinical trial was conducted

in which the MR antagonist spironolactone or a placebo was administered under controlled conditions to depressed patients who were being treated with amitriptyline (W. Hundt and colleagues, unpublished observations). The patients who were given spironolactone as an adjunct responded less favorably to amitriptyline than those who were given placebo (Fig. 8). Spironolactone activates the HPA system by blocking hippocampal MRs, as reflected by an enhanced hormonal response in the dexamethasone-CRH test in spironolactone-pretreated controls (Heuser et al., 1998). The route by which antidepressants might improve MR and GR function is unknown. Transcription factors and co-activators of the preinitiation complex involved in glucocorticoid receptor-mediated transactivation or transrepression are still unidentified drug targets.

More research was directed to antidepressant actions that involve signaling through G-protein-coupled cell membrane receptors that activate kinases. Following antidepressant treatment, the acute cellular response consists in an activation of cAMP through G-protein-mediated stimulation of adenylyl cyclase. Enhancement of cAMP-stimulated protein kinase A (PKA) results in an activation of gene transcription by the cAMP/ Ca^{2+} -responsive element (CRE) through its cognate transcription factor, the CRE-binding protein (CREB). The CRE is a DNA motif found in promoters of many genes

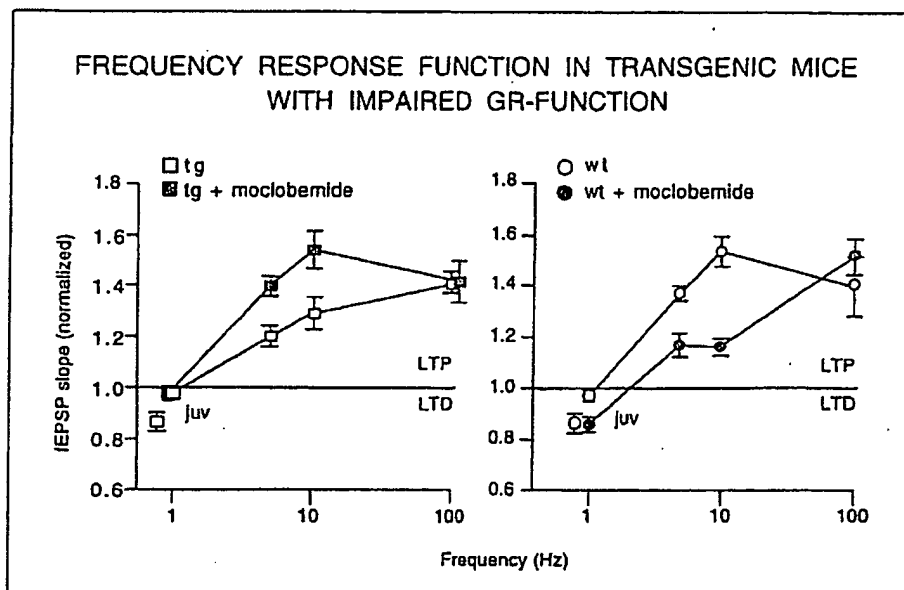


Fig. 7. Long-term potentiation (LTP) in the hippocampal area CA₁ is reduced in transgenic mice with impaired glucocorticoid receptor functions relative to the control LTP levels obtained after low frequency (5–10 Hz) stimulation. Long-term treatment of transgenic mice with the selective reversible monoamine oxidase A inhibitor moclobemide (10 mg/kg/d; p.o.; five weeks) restored LTP in mutants to levels comparable to those seen in vehicle-treated controls (left). In contrast, moclobemide decreased LTP at low frequency stimulation in control animals (from: T. Steckler, G. Rummels, Weis, W. Ziegler, and F. Holsboer: unpublished results).

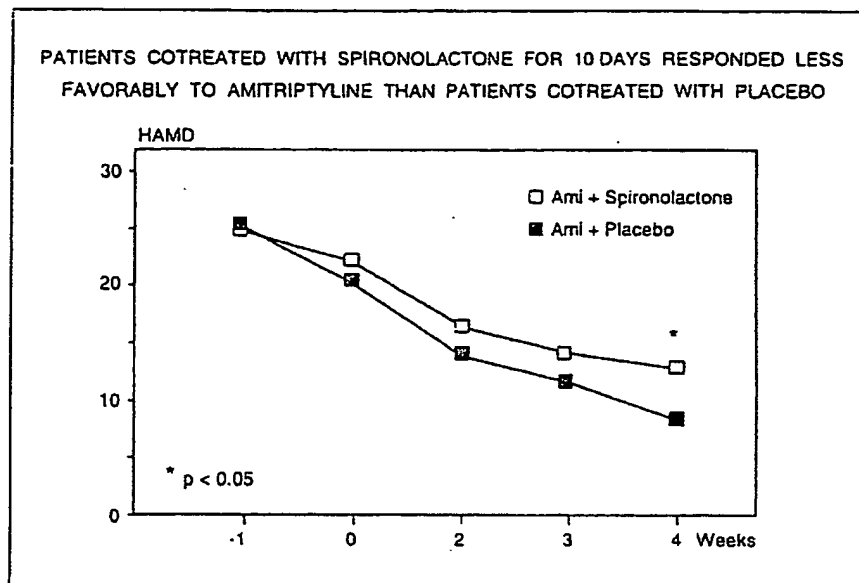


Fig. 8. Thirty patients were randomly assigned to 10 days of treatment with either 150 mg amitriptyline (Ami) plus 100 mg spironolactone (an MR antagonist) or amitriptyline plus placebo. The placebo group had a significantly better treatment outcome according to Hamilton Depression Rating Scale scores (from: W. Humdt, E. Friess, A. Ströhle, H. Reul, A. Zobel, A. Sonntag, and F. Holsboer; unpublished results).

that does not only initiate cAMP-stimulated gene transcription (Meyer and Habener 1993) but also Ca^{2+} /calmodulin-dependent protein kinase (CaM kinase)-stimulated gene transcription via an induction of membrane depolarization by Ca^{2+} inward currents that trigger the enzymatic activity. Both PKA and CaM kinase phosphorylate CREB at serine 119, which is necessary for transcriptional activation (Sheng et al. 1990). The acute effect of most antidepressants is an increase in neurotransmitters at 5-HT or norepinephrine receptors, or both, which, by activating adenylyl cyclase through G-proteins, increase the intracellular cAMP pool. Subsequently, cAMP binds to regulatory subunits of PKA, enhancing the phosphorylation of a number of PKA substrates, such as CREB, which, when phosphorylated, directs transcription of CRE-regulated genes.

After long-term administration of antidepressants, several adaptational changes occur. As first shown by Fridolin Sulser et al. (1978), prolonged exposure of the noradrenergic neuron to NA-reuptake-inhibiting antidepressants results in desensitization of β -adrenoceptors through a mechanism that has been identified only some years ago (Lefkowitz, 1993). As illustrated in Fig. 9, dissociation of the α -guanosine triphosphate (GTP) subunit from the complete $\alpha\beta\gamma$ -G-protein assembly allows the formation of a complex between the $\beta\gamma$ -dimer and the β -adrenoceptor kinase (β -ARK). This complex binds to the β -adrenoceptor and induces its phosphorylation through protein kinases in association

with β -arrestin, which terminates β -adrenoceptor-mediated signaling. This mechanism leads via decreased cAMP and subsequent decrease in PKA activation to reduced phosphorylation of substrates, such as CREB. Antidepressants also inhibit depolarization-induced Ca^{2+} influx into neuronal cells (Cai and McCaslin, 1992) and L-type Ca^{2+} currents in primary neuronal cells (Choi et al., 1992). Provided that these mechanisms also apply under pharmacotherapeutic conditions, one would expect that antidepressant-decreased Ca^{2+} influx will result in reduced phosphorylation of CaM kinase substrates, including CREB. Both antidepressant effects at the cell membrane, i.e. β -adrenoceptor stimulation and inhibition of Ca^{2+} influx, result over time in decreased CREB/CRE-directed gene transcription. These effects are most likely amplified, because three CREs within the CREB gene were identified, suggesting that the CREB gene itself is autoregulated through a CREB/CRE mechanism (Meyer and Habener, 1993).

The antidepressant-induced acute effects and long-term adaptations also have immediate and delayed effects on the HPA system, because the CRH gene expression is directed through CREB/CRE transactivation. Phi Van et al. (1990) observed stimulation of the human CRH gene promoter by cAMP in the mouse AtT 20 anterior pituitary cell line and postulated a CRE motif at position -221 base pairs, which is compatible with the finding of a cAMP-activated CRH promoter in the rat (Seasholtz et al., 1988). By progressive 5' end deletions of the human

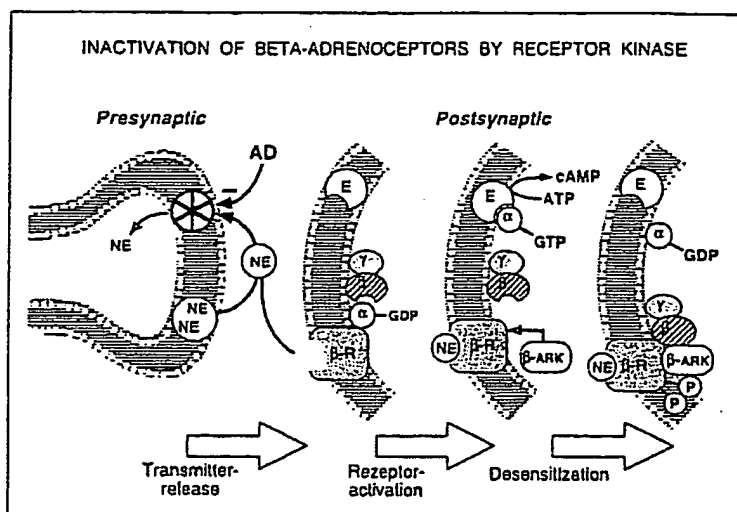


Fig. 9. Model of β -adrenoceptor 'down-regulation' through continuous ligand activation as proposed by Lefkowitz (1993). NE = norepinephrine; AD = antidepressant; β -R = β -adrenoceptor; α, β, γ = G-protein subunits; β -ARK = β -adrenoceptor kinase; GTP, GDP = Guanosine tri (di)phosphate.

CRH promoter, Spengler et al. (1992) identified a region that contained the sequence TGACGTCA at –221 base pairs, consistent with a perfect consensus motif for CRE involvement (Fig. 10). This finding explains the acute effect of antidepressants on HPA activity, which is stimulatory, as well as the long-term effects, which are suppressive (Fig. 11) (Holsboer and Barden, 1996). The suppressive effects after long-term antidepressant treatment have been well-documented by repeated administration of the dexamethasone-CRH test to patients with depression (Heuser et al., 1994; Holsboer-Trachsler et al., 1994; Zobel et al., 1999, in press) and to healthy controls (Heuser et al., 1996). Michelson et al. (1997) reported decreased plasma ACTH and cortisol responses to AVP in healthy volunteers who received imipramine at therapeutic dosages for six weeks. This finding indicates that less CRH is released endogenously after treatment and that the synergizing effect of intravenously administered AVP on ACTH secretion is consequently restricted. However, apart from this CRH-suppressive effect of antidepressants, which is well-characterized at the cellular and molecular level both in animals and humans, there are also some other adaptational responses to long-term antidepressant treatment that are of particular interest. It has been reported that chronic administration of antidepressants leads to altered binding and activation of cAMP (Nestler et al., 1989; Perez et al., 1989, 1991) and also to changes in the phosphorylation state (Racagni et al., 1992; Mori et al., 1998) and the concentrations of some PKA substrates, such as CREB (Nibuya et al., 1996). The finding of increased CREB mRNA in the rat hippocampus after three weeks of treatment with various

antidepressants has contributed to the hypothesis that antidepressants act through increases in CREB synthesis, which activate the expression of brain-derived neurotrophic factor (BDNF) in the hippocampus, thus counteracting the neurodegenerative effects of stress, which reportedly decrease BDNF (Duman et al., 1997). Although this hypothesis is appealing, it remains to be determined whether changes in constitutively expressed CREB have a functional significance of their own, or whether the phosphorylation of CREB is the only determinant event through which CREB acts on genes. Moreover, it has to be demonstrated changes in brain BDNF concentrations in the hippocampus play a causal role in depression.

In view of the findings that normalization of initial HPA hyperactivity precedes resolution of depressive symptomatology in the majority of cases, whereas continuation of HPA abnormalities is prognostically unfavorable, the question arises of how glucocorticoids interfere with the CREB/CRE-mediated gene transcription. As already mentioned, transrepression of CRH most likely occurs through protein–protein interaction, including association of ligand-activated GRs with the c-jun component of the API-complex and with other transcription factors that, without interaction with GRs, would be transactivators (Schüle et al., 1990). These transcription factors include *nur77* and CREB, and interaction of GRs with cAMP-induced CREB/CRE-directed gene transcription has been reported particularly often. Glucocorticoid-induced regulation of the somatostatin gene, for example, depends on PKA activity and may be mediated by a GR/CREB protein–protein interaction.

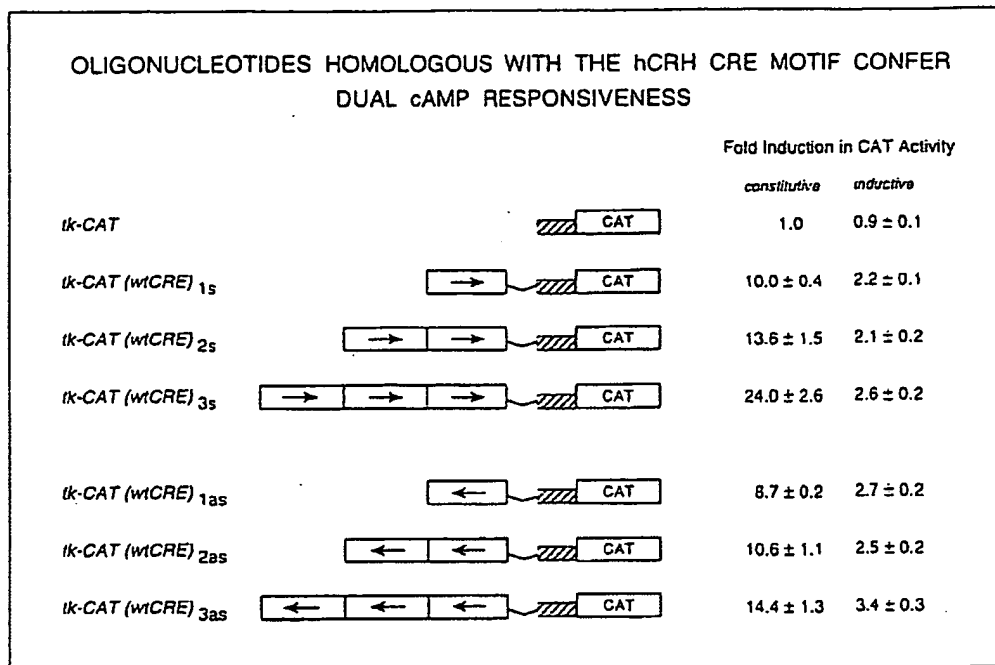


Fig. 10. An oligonucleotide (wtCRE) homologous with the sequence -238 to -216 bp of the hCRH promoter was cloned in single or multiple copies (open boxes) in forward or reverse orientation (arrows) in front of the tk promoter (hatched boxes). Constitutive expression of these constructs was referred to the parent vector (tk-CAT). Expression by treatment with 25 μ M forskolin is compared with the basal chloramphenicol acetyltransferase (CAT) activity of each construct tested. Results represent the mean \pm S.E.M. of four independent experiments expressed in terms of induction in JAR cell lines. The data show the responsiveness of the CRE in CRH gene promoter to cAMP signaling (from: Spengler et al., 1992).

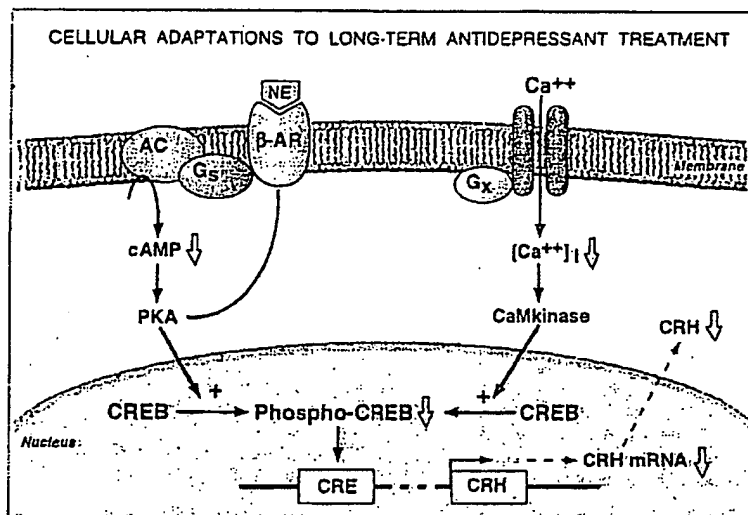


Fig. 11. Postulated effects of antidepressants leading to decreased activation (phosphorylation) of abundant cAMP-response element-binding protein (CREB) and subsequent decrease of CRH gene expression.

Similarly, GRs and cAMP amplify each other's effects on proenkephalin gene expression in C6 rat glioma cells (Joshi and Sabol, 1991), which is consistent with the mechanism suggested by the group of Ronald Evans in La Jolla, U.S.A., that PKA increases the DNA-binding activity of GR, thus upregulating corticosteroid-dependent transactivation (Rangarajan et al., 1992). Finally, investigations of P. Schmidt and colleagues (unpublished observations) recently showed that norepinephrine can enhance GR/GRE-directed gene expression through PKA-induced CREB activation. Thus, a decreased activation of both GRs and CREB after prolonged exposure to antidepressants would decrease the CREB/CRE- as well as the GR/GRE-regulated gene expression.

Of course, these effects are not uniform across cells and brain areas, and their significance for the clinical condition remains elusive. More directly related to counteracting the clinical effects of antidepressants is perhaps the finding of the group of Lefkowitz that β -adrenoceptors are transcriptionally regulated by glucocorticoids (Collins et al., 1988), which confirms earlier work by Mobley and Sulser (1980) showing that adrenoceptor agonist-induced cAMP accumulation in brain slices is controlled by corticosteroids. If this mechanism is also involved in the clinical condition of depression, the sustained activation of HPA secretion would counteract the antidepressant-induced desensitization of adrenoceptors and the subsequent decrease in intracellular signaling that regulates CREB/CRE-directed genes, including CRH.

Taken together, the reported basic research findings are consistent with the clinical observations and animal experiments which propose that antidepressants act through enhancing corticosteroid receptor function and repressing CRH gene activity. As many of the conclusions that have been drawn are weakened by the artificiality of the test systems from which they were derived, there is an obvious need to show that the *in vitro* findings are also valid at the organismic levels in animals.

7. CRH receptors

The CRH signal is mediated through functionally and regionally different cell membrane receptors. Up to now, two CRH receptors have been identified, which comprise seven putative transmembrane helices and belong to the family of G_i -protein-coupled receptors (Fig. 12). They are encoded by two distinct genes, both of which have been identified (Chalmers et al., 1996). The CRH₁ receptor was identified and cloned from a human ACTH-secreting pituitary adenoma (Chen et al., 1993), mouse pituitary (Vita et al., 1993), rat brain (Perrin et al., 1993) and human brain (Chang et al., 1993). Species homologues show 98% sequence identity over their full length of 415 amino acids. The CRH₁ receptor has five *N*-linked

glycosylation sites in the N-terminal extracellular domain and at least two potential phosphorylation sites for protein kinase C (PKC) in the first and second extracellular loop and in the C-terminal tail. Chen et al. (1993) identified an alternatively spliced form of the CRH₁ receptor in human pituitary, the functional significance of which remains to be determined. The regional and cellular distribution of CRH₁ and CRH₂ receptors is given in Table 1 and Fig. 13. It indicates that the CRH₁ receptor is very highly expressed in the cerebral cortex, the amygdala, the hippocampus, the cerebellum and the pituitary. All forms of CRH₁ receptors show a homology of about 30% to other neuropeptide receptors, including those for pituitary adenylyl cyclase-activating peptide, growth hormone-releasing hormone and glucagon. The expression pattern is consistent with a role of CRH₁ receptors in mediating not only the neuroendocrine but also the behavioral and autonomic responses to stress. When expressed in stable cell lines, human/rat CRH₁ receptors show reversible, saturable and high-affinity binding of CRH, eliciting cAMP production with an EC of ≈ 1 nM.

Two different forms of the CRH₂ receptor, CRH_{2a} and CRH_{2b}, have been identified in rodents, both forms having been produced by alternative splicing (Lovenberg et al., 1995; Stenzel et al., 1995). Each has a distinct distribution and function. The CRH_{2a} receptor is found predominantly in neurons and a 411-amino acid protein that shares about 71% sequence identity with the CRH₁ receptor. The amino acid differences are located in extracellular domains, forming the ligand-binding structure, whereas the sequence identity is particularly pronounced between transmembrane domains 5 and 7 (Dautzenberg et al., 1997, 1998). These domains are involved in G_s -protein-coupled activation of the cAMP/PKA pathway, and both the CRH₁ and CRH₂ receptor forms activate this G_s -protein-coupled signaling. The localization of CRH_{2a} receptors is distinct from that of CRH₁ receptors, CRH_{2a} receptors being expressed in limited areas of the brain, including the lateral septal nucleus, the ventromedial hypothalamic nucleus and the medial amygdaloid nucleus. In rodents, the CRH_{2b} variant predominates in the brain, whereas the CRH_{2a} subtype is confined to nonneuronal structures such as the choroid plexus, cerebral blood vessels and peripheral structures in cardiac skeletal muscle, and, at lower levels, the lung and intestine (Lovenberg et al., 1995; Perrin et al., 1995).

In humans, in contrast to rodents, CRH₂ receptors exist in three different functional splice variants (α , β , γ). The CRH_{2a} and CRH_{2b} receptor variants are coexpressed in the brain and periphery (Valdenaire et al., 1997), whereas the CRH_{2c} receptor subtype seems to be expressed only in the brain, mainly in septum and hippocampus (Kostich et al., 1998). These significant differences in CRH₂ receptor expression between rodents and humans may limit extrapolations of CRH₂ receptor functions from rat to human physiology. The most potent ligand

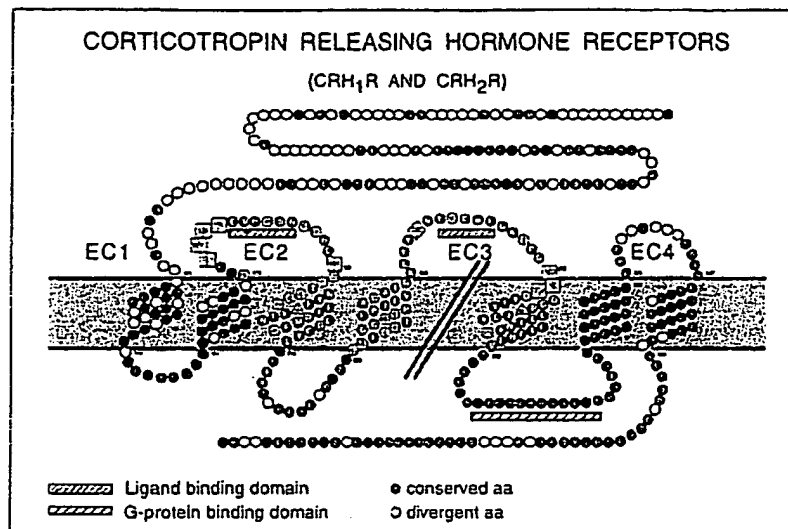


Fig. 12. CRH₁ and CRH₂ receptor structure with high conservation in the seven transmembrane loops and the intracellular tail. The double-bar indicates that a 3.1-kb fragment of the CRH₁ receptor encoding the transmembrane domains 4–7 (including the G-protein-coupling domain and the intracellular tail) was replaced by a phosphoglycerate kinase promoter-driven neomycin cassette (Timpl et al., 1998). This intervention leaves the ligand-binding domains in place, though their binding capacity is diminished.

of CRH₂ receptors expressed in stable cell lines is urocortin, a urotensin-like peptide isolated from the Edinger-Westphal nucleus that has 45% sequence identity to CRH and has been implicated mainly in the regulation of appetite (Vaughan et al., 1995; Spina et al., 1996). The view that urocortin is the preferred mammalian ligand for central CRH₂ receptors is supported by the finding of Wang et al. (1996) that systemic administration of urocortin specifically activates neurons in the supraoptic nucleus (SON), the ventromedial hypothalamic nucleus and the magnocellular part of the hypothalamic paraventricular nucleus. Administration of urocortin induces more *fos* expression in neurons in the SON and magnocellular PVN than CRH, which is compatible with a CRH₂ receptor-mediated effect because in these structures CRH₂ receptor expression is very high. These regional and functional studies suggest that behavioral effects mediated by CRH in the structures mentioned also involve CRH₂ receptors.

Following CRH exposure, CRH₁ receptors interact with G-proteins to activate cAMP in a dose-dependent manner, which in turn activates cAMP protein kinases (Fig. 14). In addition, CRH receptors have potential PKC phosphorylation sites. PKC is activated by Ca²⁺ in conjunction with diacylglycerol, a second messenger produced by phospholipase C, which catalyzes the breakdown of phosphatidylinositol into diacylglycerol and inositol triphosphate (Duman and Nestler, 1995). According to earlier studies, CRH can modulate the Ca²⁺-dependent ion currents (Aldenhoff et al., 1983) and

intracellular Ca²⁺ increases may be dependent on CRH receptor-linked calcium channels (Takuma et al., 1994). Through these mechanisms the PKC pathway may become activated, which is important for understanding the synergy between CRH and vasopressin. The latter ACTH secretagogue, produced mainly in magnocellular neurons under basal conditions and coexpressed in parvocellular neurons under chronic stress, is a direct activator of PKC through V_{1R} receptors. Thus, the potentiating effect on activation of the POMC gene expression stems from crosstalk between PKC and cAMP-dependent kinases.

CRH₁ receptors at pituitary corticotrophs are desensitized in response to enduring agonist exposure. This desensitization is homologous as it does not affect cAMP accumulation in response to stimulation of other G_s-protein-coupled receptors. Not only long-term CRH administration but also adrenalectomy produces CRH₁ receptor desensitization at corticotrophs. The latter finding is consistent with the CRH-suppressive effect of corticosteroids on hypothalamic CRH gene expression through transrepression via protein–protein interaction (see above, and Aguilera, 1994; Rabadan-Diehl et al., 1997). A direct effect of high corticosteroid doses at pituitary cells has been reported to decrease CRH binding, but the functional significance of this effect has yet to be elucidated. According to a model proposed by the group of Lefkowitz (Fig. 9), G-protein-coupled receptors such as β -adrenoceptors are desensitized by PKA phosphorylation of residues in the third intracellular loop.

Table 1
Semi-quantitative evaluation of CRH₁ and CRH₂ receptor mRNA distribution in rat brain and pituitary gland (from Chalmers et al., 1995)

Anatomical region	mRNA abundance	
	CRH ₁	CRH ₂
Telencephalon		
Olfactory bulb:		
External granular layer	++	—
Internal granular layer	++++	++
Mitral cell layer	++++	—
Ependyma	++	+++
Accessory olfactory nucleus	++	++
Frontal cortex (superficial)	+++	—
Frontal cortex (deep)	+++	—
Cingulate cortex (superficial)	+++	—
Cingulate cortex (deep)	+++	—
Lateral septum (ventral)	+	++++
Lateral septum (intermediate)	+	++++
Medial septum	++	±
Bed nucleus of the strium terminalis (medial)	++	++
Amygdala		
Basolateral nucleus	++++	±
Medial nucleus	++++	++
Posterior cortical	+	+++
Hippocampus		
CA1	++	++
CA3/4	++++	++
Dentate gyrus	++	++
Entorhinal cortex	++	++
Diencephalon		
Hypothalamus		
Paraventricular nucleus	±	++
Supraoptic nucleus	+	+++
Lateral hypothalamus	+	+
Dorsomedial hypothalamus	+++	—
Ventromedial hypothalamic nucleus	+	++++
Medial geniculate nucleus	++	±
Mesencephalon		
Superior colliculus (superficial layer)	+++	+
Interpeduncular nucleus	++++	+++
Dorsal raphe nucleus	+	++
Caudal linear nucleus	+	+++
Red nucleus	++++	—
Pons/medulla		
Inferior colliculus	++	++
Lateral dorsal tegmental nucleus	++++	—
Locus coeruleus	—	—
Cerebellar cortex	++++	±
Pontine gray	++++	±
Motor trigeminal nucleus	++++	—
Sensory trigeminal nucleus	+++	±
Choroid plexus	—	++++
Pituitary gland		
Anterior lobe	+++	±
Intermediate lobe	+++	±
Posterior lobe	—	—

CRH₁ and CRH₂ mRNA abundance for each anatomical region was determined from optical density measurements. Density values for each parameter are presented according to their respective percentile distributions: + + + + (> 75%), very dense: + + + (< 75%, > 50%), dense: + + (< 50%, > 25%), moderate: + (< 25%, > 0%), low: ± (< 10%), scattered cells.

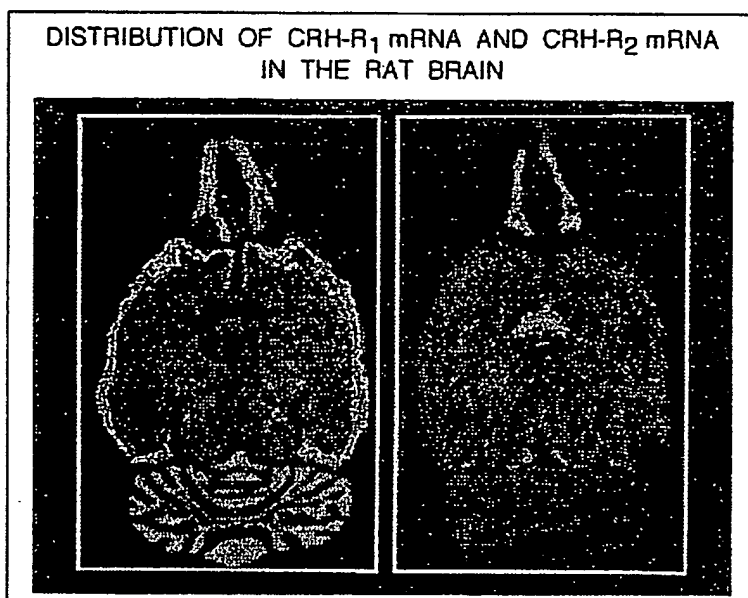


Fig. 13. CRH-R₁ and CRH-R₂ distribution as analysed by *in situ* hybridization (see also Table 1) suggests that both receptor subtypes mediate different effects by their ligands CRH and urocortin (Chalmers et al., 1995).

Whether this mechanism also applies to CRH receptors remains open, particularly because the time needed for reinstatement of receptor function is much longer than those reported for adrenoceptors (Hauger et al., 1997).

CRH₁ receptor adaptation to agonist stimulation in the brain is much less well studied. Following central CRH administration, CRH receptors were desensitized in the amygdala (Hauger and Aguilera, 1993; Hauger et al., 1993) and in the frontal cortex. In line with this is a recent study showing that acute stress elicits CRH mRNA in the central amygdala (Hsu et al., 1998), whereas long-term stress or CRH administration upregulates CRH receptors in the hippocampus, hypothalamic PVN and SON (Luo et al., 1994; Iredale et al., 1997). Down-regulation of CRH receptors in the amygdala and frontal cortex after chronic unpredictable stress may be a consequence of elevated CRH secretion under these conditions. In contrast, CRH₁ receptor mRNA was found to be increased in the hippocampus and hypothalamic PVN in response to unpredictable stress (Iredale et al., 1996). Similarly, an immune challenge and immobilization stress were reported to enhance CRH₁ receptor mRNA, which, provided that translation into receptor protein occurs, leads to enhanced CRH responsiveness in these brain regions under stress (Rivest et al., 1995). The mechanism underlying the CRH-induced upregulation of CRH₁ receptors in the hippocampus and hypothalamus may represent a feed-forward loop that maintains the organism's capacity to respond to an acute stressor under con-

ditions of chronic stress. Elucidation of the mechanism by which CRH upregulates or downregulates CRH receptors in the brain requires characterization of the promoter region of the CRH₁ receptor gene and its cell-specific regulation.

8. Preventing CRH actions by blocking its receptors

Given the evidence that the neuropeptide CRH, when hypersecreted continuously in rats, produces numerous behavioral changes resembling the cardinal symptoms of depression and anxiety, the most straightforward therapeutic strategy is a blockade of its action by CRH receptor antagonists. The first CRH receptor antagonist described (Rivier et al., 1984) was the α -helical CRH₄₁₋₄₁ peptide, an N-terminus-shortened analog of human/rat CRH. This molecule proved to be a competitive inhibitor of CRH-elicited ACTH secretion in pituitary cells and has been subsequently studied in a large number of behavioral experiments in rats. These studies, mainly conducted in the laboratory of George Koob in La Jolla, U.S.A., consistently showed that α -helical CRH₄₁₋₄₁ suppresses behavioral and neuroendocrine responses to CRH administration (Britton et al., 1986; Heinrichs et al., 1992; Koob et al., 1993), and to various emotional stressors (Heinrichs et al., 1994; Korte et al., 1994). The α -helical CRH₄₁₋₄₁ peptide has also been administered to humans and has been shown to block pituitary CRH

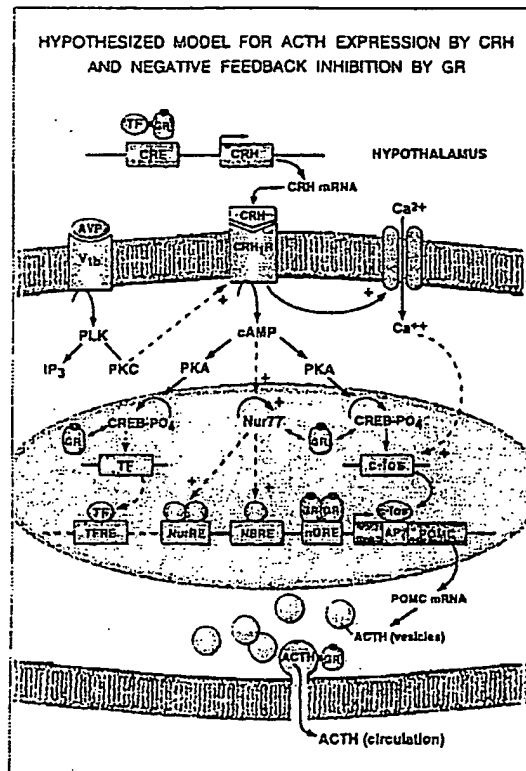


Fig. 14. This scheme explains how ACTH and corticosteroid secretion are maintained in the absence of a functional CRH₁ receptor through activation of the POMC gene via orphan receptors (*nur77*) which trans-activate via response elements (NurRE, NBRE) that require either *nur77* monomers or dimers. Other secretagogues released from the hypothalamus may also activate POMC gene expression through non-CRH₁ receptor-mediated processes. Activation of CRH₁ receptors through CRH results in enhanced *nur77* activation and a further activation of a variety of transcription factors (TF, *c-fos*) which amplify each other in enhancing POMC gene expression. Counterregulation requires dimerization of ligand-activated glucocorticoid receptors and subsequent DNA binding at negative response elements (nGRE).

receptors, as evidenced by decreased ACTH and cortisol secretion (Baran et al., 1996a). The peptide was given as an intravenous infusion, and the absence of anxiolytic effects was attributed to its inability to cross the blood-brain barrier. However, the individuals studied were young healthy controls without any signs of psychopathology. The anxiolytic effects of central CRH receptor blockade are to be expected only if CRH secretion is elevated in pathological conditions during severe stress, which is never the case in healthy individuals at rest. Nevertheless, a possible central effect of peripherally administered α -helical CRH₄₁ peptide cannot be excluded as peripherally administered neuropeptides, provided the peptide is given in a pulsatile mode, can

have distinct CNS effects, for example on sleep (Steiger and Holsboer, 1997).

One major disadvantage of α -helical CRH₄₁ is that it showed CRH-like weak intrinsic agonistic ('stress-like') activity in some but not all in vitro and in vivo studies (Baldwin et al., 1991; Rainnie et al., 1992; Wiersma et al., 1995). A new CRH-derived peptide in which the N-terminus was shortened in addition to several amino acid substitutions, D-Phe¹², Nle^{21,28}, (α MeLeu²⁷) CRH₁₂₋₄₁ (abbreviated D-Phe CRH₁₂₋₄₁), was introduced by Menzaghi et al. (1994) and shown to block CRH-induced behavioral responses such as locomotor activation five times more potently than α -helical CRH₄₁, which is consistent with the in vitro data demonstrating that D-Phe CRH₁₂₋₄₁ was eight times more potent in preventing CRH-induced ACTH secretion. In addition to its higher potency, D-Phe CRH₁₂₋₄₁ proved to lack intrinsic agonistic effects (Menzaghi et al., 1994). In the same laboratory D-Phe CRH₁₂₋₄₁ was used to test whether the anxiogenic-like effect of cannabinoid receptor stimulation by the synthetic agonist HU 210 could be blocked by CRH antagonists (Rodriguez de Fonseca et al., 1996). Cannabinoids activate the HPA system, and the associated anxiogenic effects are likely to be mediated by CRH receptors as D-Phe CRH₁₂₋₄₁ is capable of attenuating the behavioral effect. Systemic cocaine administration produced a conditional saccharine aversion, which was dose-dependently potentiated by central administration of D-Phe CRH₁₂₋₄₁, implicating CRH activation in cocaine-related motivational states, too (Rodriguez de Fonseca et al., 1996, 1997).

Neither α -helical CRH₄₁ nor D-Phe CRH₁₂₋₄₁ possesses the property to selectively bind to different CRH-receptor subtypes, but a drug with a potential for clinical efficacy needs to be targeted against specific CRH receptors in order to limit side effects. One strategy for delineating which CRH receptor subtype mediates CRH-induced psychopathology consists in using antisense probes. Liebsch et al. (1995) infused an antisense ODN corresponding to the CRH₁ receptor mRNA bilaterally into the central amygdala of rats for four days prior to subjecting the animals to social defeat and subsequent testing of anxiety-related behavior. As illustrated in Fig. 15, ODNs corresponding to the CRH₁-receptor mRNA had anxiolytic effects in rats. These findings were compatible with those obtained in studies using ODNs corresponding to CRH mRNA (Skutella et al., 1994a,b) and in a study by Swiergiel et al. (1993) showing that stress-induced behavior is attenuated after CRH receptor antagonist infusion into the central amygdala. In a subsequent study, Skutella et al. (1998) investigated extensively the effects of an antisense probe corresponding to CRH₁ receptor mRNA in vitro and in vivo. The antisense probe reduced CRH binding and function, as measured by ACTH secretion, in primary rat anterior pituitary cells and in clonal mouse pituitary tumor cells (AtT 20), and

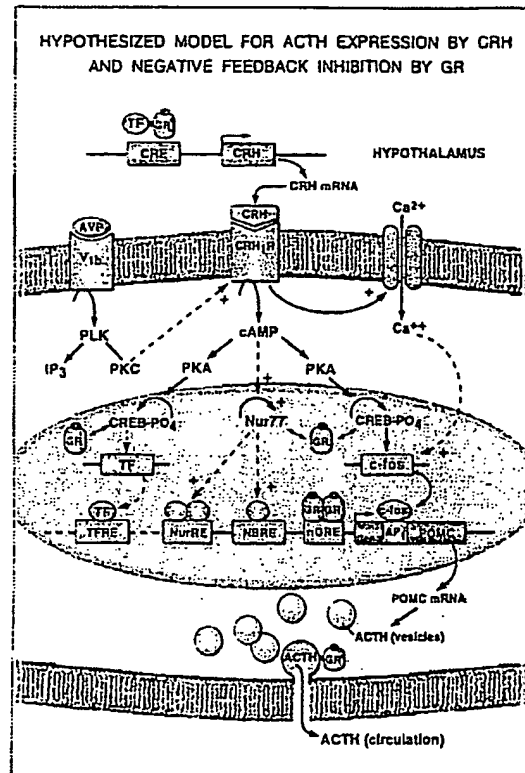


Fig. 14. This scheme explains how ACTH and corticosteroid secretion are maintained in the absence of a functional CRH₁ receptor through activation of the POMC gene via orphan receptors (*nur77*) which transactivate via response elements (NurRE, NBRE) that require either *nur77* monomers or dimers. Other secretagogues released from the hypothalamus may also activate POMC gene expression through non-CRH₁ receptor-mediated processes. Activation of CRH₁ receptors through CRH results in enhanced *nur77* activation and a further activation of a variety of transcription factors (TF, *c-fos*) which amplify each other in enhancing POMC gene expression. Counterregulation requires dimerization of ligand-activated glucocorticoid receptors and subsequent DNA binding at negative response elements (nGRE).

receptors, as evidenced by decreased ACTH and cortisol secretion (Baram et al., 1996a). The peptide was given as an intravenous infusion, and the absence of anxiolytic effects was attributed to its inability to cross the blood-brain barrier. However, the individuals studied were young healthy controls without any signs of psychopathology. The anxiolytic effects of central CRH receptor blockade are to be expected only if CRH secretion is elevated in pathological conditions during severe stress, which is never the case in healthy individuals at rest. Nevertheless, a possible central effect of peripherally administered α -helical CRH₉₋₄₁ peptide cannot be excluded as peripherally administered neuropeptides, provided the peptide is given in a pulsatile mode, can

have distinct CNS effects, for example on sleep (Steiger and Holsboer, 1997).

One major disadvantage of α -helical CRH₉₋₄₁ is that it showed CRH-like weak intrinsic agonistic ('stress-like') activity in some but not all in vitro and in vivo studies (Baldwin et al., 1991; Rainnie et al., 1992; Wiersma et al., 1995). A new CRH-derived peptide in which the N-terminus was shortened in addition to several amino acid substitutions, D-Phe¹², Nle^{21,25}, (zMeLeu³⁷) CRH₁₂₋₄₁ (abbreviated D-Phe CRH₁₂₋₄₁), was introduced by Menzaghi et al. (1994) and shown to block CRH-induced behavioral responses such as locomotor activation five times more potently than α -helical CRH₉₋₄₁, which is consistent with the in vitro data demonstrating that D-Phe CRH₁₂₋₄₁ was eight times more potent in preventing CRH-induced ACTH secretion. In addition to its higher potency, D-Phe CRH₁₂₋₄₁ proved to lack intrinsic agonistic effects (Menzaghi et al., 1994). In the same laboratory D-Phe CRH₁₂₋₄₁ was used to test whether the anxiogenic-like effect of cannabinoid receptor stimulation by the synthetic agonist HU 210 could be blocked by CRH antagonists (Rodriguez de Fonseca et al., 1996). Cannabinoids activate the HPA system, and the associated anxiogenic effects are likely to be mediated by CRH receptors as D-Phe CRH₁₂₋₄₁ is capable of attenuating the behavioral effect. Systemic cocaine administration produced a conditional saccharine aversion, which was dose-dependently potentiated by central administration of D-Phe CRH₁₂₋₄₁, implicating CRH activation in cocaine-related motivational states, too (Rodriguez de Fonseca et al., 1996, 1997).

Neither α -helical CRH₉₋₄₁ nor D-Phe CRH₁₂₋₄₁ possesses the property to selectively bind to different CRH-receptor subtypes, but a drug with a potential for clinical efficacy needs to be targeted against specific CRH receptors in order to limit side effects. One strategy for delineating which CRH receptor subtype mediates CRH-induced psychopathology consists in using antisense probes. Liebsch et al. (1995) infused an antisense ODN corresponding to the CRH₁ receptor mRNA bilaterally into the central amygdala of rats for four days prior to subjecting the animals to social defeat and subsequent testing of anxiety-related behavior. As illustrated in Fig. 15. ODNs corresponding to the CRH₁-receptor mRNA had anxiolytic effects in rats. These findings were compatible with those obtained in studies using ODNs corresponding to CRH mRNA (Skutella et al., 1994a,b) and in a study by Swiergiel et al. (1993) showing that stress-induced behavior is attenuated after CRH receptor antagonist infusion into the central amygdala. In a subsequent study, Skutella et al. (1998) investigated extensively the effects of an antisense probe corresponding to CRH₁ receptor mRNA in vitro and in vivo. The antisense probe reduced CRH binding and function, as measured by ACTH secretion, in primary rat anterior pituitary cells and in clonal mouse pituitary tumor cells (AtT 20), and

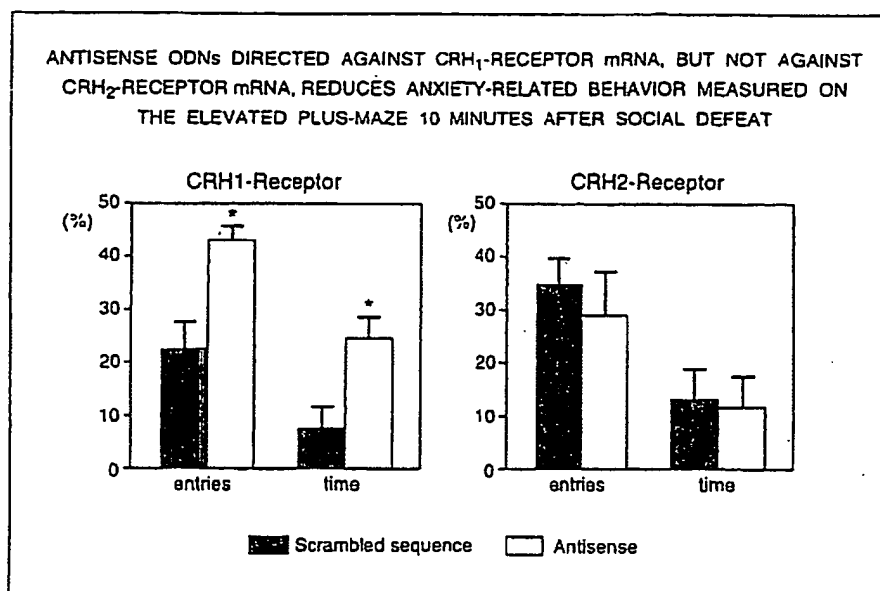


Fig. 15. In the elevated plus-maze test socially defeated rats show less anxiety-related behavior (more entries and more time spent on open arms) if chronically pretreated with an antisense probe directed against the CRH₁, but not against the CRH₂, receptor mRNA (see Table 2, from: Liebsch et al., 1999, in press).

provided evidence that this action is associated with cytoplasmatic uptake of the probe.

Intracerebroventricular infusions of antisense, sense and mismatch probes to rats confirmed that inhibition of CRH₁ receptor mRNA translation into CRH₁ receptor protein reduces CRH-elicited anxiety-related behavior in rats. This effect was associated with decreased CRH binding in the hypothalamus and cortex (Skutella et al., 1998). The localization of CRH₂ receptors suggests that these receptors are also involved in the mediation of CRH-induced behavioral changes. This notion is supported by a study by Moreau et al. (1997), who showed that urocortin, which acts primarily through CRH₂ receptors, is an anxiogenic neuropeptide that also has an anorexic effect. Therefore, Liebsch et al. (1999, in press) compared the effects of antisense probes directed against CRH₁ and CRH₂ receptors. When these probes were infused chronically into the lateral ventricle of rats, partial loss of function of CRH₁ and CRH₂ receptors produced distinct behavioral effects (Table 2). As expected, there was an anxiolytic effect in animals treated with CRH₁ receptor antisense ODN, whereas no such effect was observed in those treated with CRH₂ receptor antisense ODN, thus confirming findings obtained by Heinrichs et al. (1997). However, the CRH₂ receptor antisense treatment increased immobility in a forced swim test, which suggests that the CRH₂ receptor plays a role in stress-coping behavior.

According to a widespread interpretation of the forced

swim test, (also known as the Porsolt test), reduced immobility is believed to be indicative of an antidepressive potential of the applied drug. However, this is unlikely in the context of CRH receptors and has been shown previously to be unlikely in the context of moclobemide-treated transgenic mice with impaired GR function (Montkowski et al., 1995). The studies with peptides that antagonize both of the CRH receptors and antisense probes that selectively reduce CRH receptor subtype levels indicate that CRH₁ receptors are the primary target at which selective nonpeptide compounds designed to treat anxiety and stress-related disorders should be directed.

Several drug companies have taken up this concept and employed high-speed screening of compound libraries yielding lead compounds that after chemical modifications have fulfilled the criteria for specific CRH₁ receptor antagonists. Most of these studies are unpublished for patent reasons, and therefore this report is limited to compounds the companies do not plan to take up into clinical development programs.

Schulz et al. (1996) reported that a pyrrolo[2,3-d]pyrimidine derivative, CP-154,526, produced by Pfizer Inc., binds selectively to the CRH₁ receptor subtype (Fig. 16). This compound readily enters the CNS after peripheral administration. Using increases in acoustic startle produced by icv CRH as an indicator of a CRH-elicited increase in fear and anxiety, the group found that CP-154,526 blocked the CRH effects completely. Firing

Table 2
Effect of CRH₁ and CRH₂ receptor knockdown

Test	Day	CRH ₁ receptor	CRH ₂ receptor
Social discrimination (olfactory memory)	3	no effect	no effect
Elevated plus-maze (anxiety)	4	anxiolytic	no effect
Open field (locomotor activity)	4	no effect	no effect
Forced swim test (stress coping)	5/6	no effect	increased immobility

Antisense oligodeoxynucleotides were administered intracerebroventricularly through osmotic minipumps at a rate of 5 µg/hr to male Wistar rats for seven days. Under these experimental conditions only CRH₁ receptor decrease had anxiolytic effects (Liesch et al., 1999, in press).

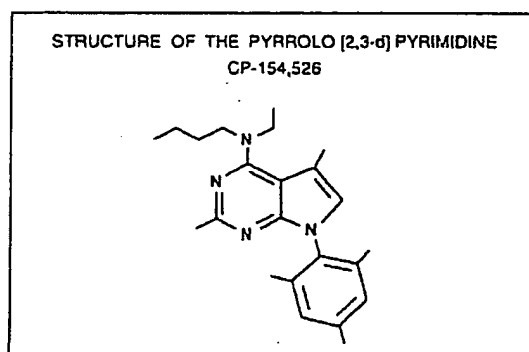


Fig. 16. High speed screening of compound libraries with a radioligand binding assay led to the discovery of a low affinity-lead compound, which after several chemical modifications resulted in the discovery of CP-154,526 (butyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine-4-yl]ethylamine), which has high affinities to cerebral cortical ($K_i = 5.7$ nM) and pituitary ($K_i = 1.4$ nM) membranes of rats (Schulz et al., 1996).

activity of the LC is a rapid response to anxiogenic exposures, activating noradrenaline release in many brain areas. As mentioned earlier, CRH activates the firing rate of the LC when directly injected or when administered icv, which is consistent with the presence of CRH-immunoreactive fibers and intersynaptic connections in this brainstem nucleus. When CP-154,526 is administered orally prior to CRH administration, the CRH-induced firing can be attenuated in a dose-dependent fashion. Lundkvist et al. (1996) showed anxiolytic effects of CP-154,526 employing the elevated plus-maze test. In an extensive study, Griebel et al. (1998) administered a large battery of behavioral tests to rats and mice and compared the anxiolytic effects of CP-154,526 with those of buspirone (a partial 5-HT_{1A} agonist) and diazepam. The overall conclusion was that this nonpeptide CRH₁ receptor antagonist has anxiolytic effects in stressed rodents. Because anxiety and exposure to stress are thought to contribute to drug-seeking behavior in humans and experimental animals, Shaham et al. (1998) performed a study with CP-154,526 in which they documented that

this drug can prevent the stress-induced reinstatement of cocaine- and heroin-taking in rodents in which these behaviors have been extinguished.

Because of the potential role of CRH in precipitating and maintaining depression, a preliminary report by Mansbach et al. (1997) is of note, in which it is postulated that CP-154,526 has antidepressant effects in rats that have been exposed to a series of inescapable foot shocks and then tested in a shock-escape test. Animals that are exposed to inescapable shocks perform poorly in the shock-escape procedure because of 'learned helplessness' (Wilner, 1984). This procedure is frequently used as an animal model of depression, and it has been shown that the acquisition deficits that develop in response to uncontrollable shock exposure, for instance in the form of such inability to escape from noxious stimuli, can be reversed by antidepressants (Wilner, 1984). When CP-154,526 was given prior to the escape test, it reversed the animals' deficit in perceiving and using the possibility to escape dose-dependently and much faster than imipramine, which needs to be administered repeatedly to achieve the same effects. Another pyrrolo[2,3-d]pyrimidine derivative (NBI 27914) was synthesized by Chen et al. (1996). This compound binds specifically to CRH₁ receptors and suppresses CRH-induced ACTH release in vitro and in vivo (Webster et al., 1996). CRH administration leads to seizures that originate in the amygdala and spread to the hippocampus and other limbic structures. These seizures occur at dosages that do not activate the HPA system (Baram et al., 1992) and are prevented by nonselective CRH receptor antagonists, but not by glutamate receptor antagonists (Baram et al., 1996b). In rat pups pretreated with NBI 27914, the duration of CRH-induced seizures can be shortened in a dose-dependent fashion (Baram et al., 1997). Other CRH₁ receptor-specific pyrrolo[2,3-d]pyrimidine derivatives also have anxiolytic effects in the rat line bred for high anxiety by Rainer Landgraf in Munich, which supports the view that these substances comprise a new class of compounds with a remarkable potential for clinical use in pathological anxiety and other stress related disorders (M. Keck and colleagues, unpublished data).

9. Mice lacking a functional CRH₁ receptor

A further set of experiments suited to complement the studies with antisense probes and antagonists targeted to the CRH₁ receptor uses mice with deficient CRH₁ receptors due to homologous recombination in embryonic stem cells. By deleting the coding sequences of the transmembrane regions V, VI and VII, including the G-protein coupling domain and the intracellular cytoplasmic tail (Fig. 12), a research team in Munich, led by Wolfgang Wurst, generated a mouse with a truncated protein instead of a functional CRH₁ receptor and hence unable to activate cAMP in response to CRH (Timpl et al., 1998). In cultured pituitary cells obtained from heterozygous mutants and homozygous mutants CRH evoked a decreased ACTH response, whereas forskolin, which directly activates the catalytic subunit of adenylyl cyclase, evoked a much more pronounced activation of ACTH (Fig. 17). In contrast to wild-type mice, in homozygous and heterozygous mutants CRH did not elicit a marked cAMP increase, which indicates that the mutations specifically impaired CRH/CRH₁ receptor signaling. Slightly different results emerged from *in vivo* studies, in which the basal plasma ACTH concentrations of homozygous and heterozygous mutants and wild-type mice proved to be indistinguishable, irrespective of gender (Fig. 18).

This discrepancy can be attributed to the presence of other ACTH secretagogues under *in vivo* conditions, where CRH₁ receptor-independent activation of the POMC gene, e.g., through *mur77* (Drouin et al., 1998), also occurs to maintain basal activity (Fig. 14). Under

stress conditions such as the forced swim test, plasma ACTH levels rise steeply in wild-type and heterozygous mice, whereas homozygous null mutants show decreased plasma ACTH levels. Similarly, plasma corticosterone concentrations are decreased in mice with CRH₁ receptor deficiency (Fig. 18). When CRH is administered to rodents or when a mouse overexpresses CRH through a transgene insertion, these animals display reduced exploratory behavior and increased anxiety (Stenzel-Poore et al., 1992, 1994; Koob et al., 1994). When tested in an open field test or in a light-dark box, mice lacking CRH₁ receptor function showed less anxiety-related behavior. These findings are in agreement with the histological and immunohistochemical data obtained from these mice (Figs 19, 20). No morphological changes could be detected in any of the brain areas investigated and no changes in other proteins involved in HPA regulation, including CRH₂ receptors, CRH-binding protein, MRs, GRs, and brain-derived neurotrophic factor, were observed. The only changes seen were elevations of CRH in the PVN, hippocampus, amygdala and cerebral cortex, which can be interpreted as adaptive responses to CRH₁ receptor deficiency.

Similar results were obtained by Smith et al. (1998), who generated a CRH₁ receptor-deficient mouse by replacing the last 12 amino acids of the first extracellular domain through the fourth transmembrane domain with a neomycin-resistant gene cassette, which resulted in a non-functional CRH₁ receptor protein. These mice were tested by using the dark-light emergence task and the elevated plus-maze test, in both of which they displayed markedly reduced anxiety-related behavior. The investigators stud-

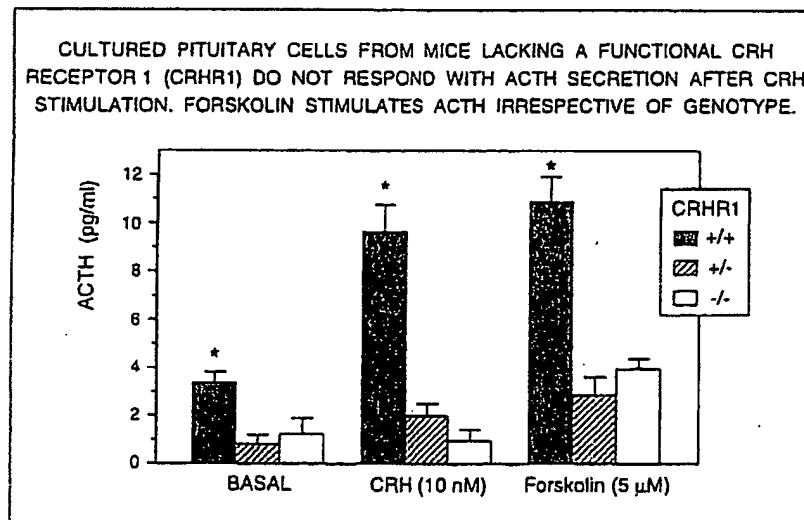


Fig. 17. In a mouse mutant that lacks a functional CRH₁ receptor ACTH release from cultured corticotrophs is decreased after CRH stimulation (adapted from Timpl et al., 1998).

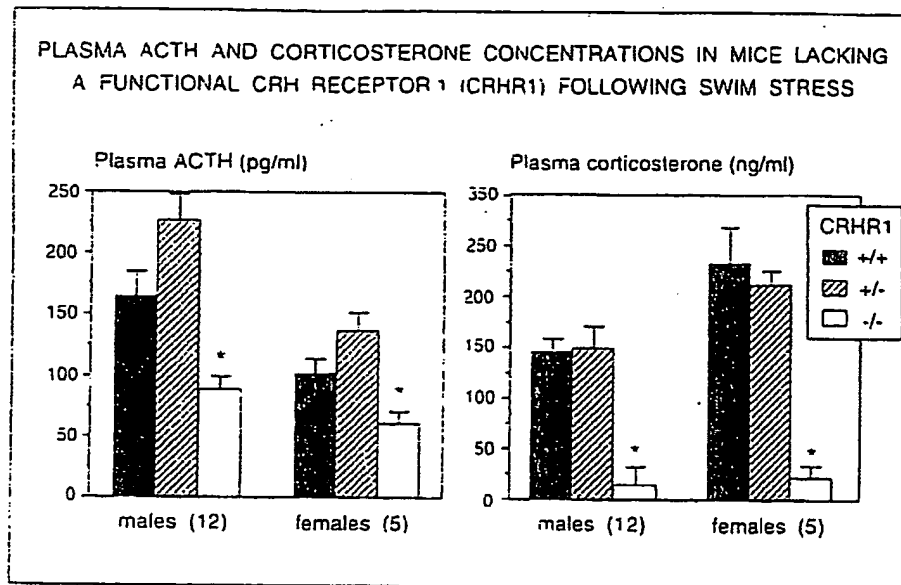


Fig. 18. In vivo studies showed that the plasma ACTH levels of homozygous and heterozygous CRH₁ receptor knockout mice and wild-type controls were indistinguishable. After forced swim stress, plasma ACTH concentrations were significantly increased in wild-type and heterozygous male and female mice, but not in homozygous mutants. Plasma corticosterone responses were also much lower in CRH₁ receptor-deficient mutants (adapted from: Timpl et al., 1998).

ied the adrenocortical changes in the CRH₁ receptor null mutants extensively and concluded that adrenal deficiency in these mice is due to decreased plasma ACTH concentrations during neonatal life (Smith et al., 1998).

One of the most severe stressors in rodents is withdrawal from alcohol, which results in excessive activation of the HPA system and strongly increased anxiety. In rodents, and most likely also in humans under clinical conditions, both phenomena are related to increased release of CRH from central neurons (von Bardeleben et al., 1989; Rassnick et al., 1993). CRH₁ receptor mutants and wild-type mice were subjected to a forced alcohol drinking procedure and were subsequently tested under withdrawal conditions (Timpl et al., 1998). In these investigations a CRH overactivity, mediated via CRH₁ receptors, could be demonstrated. When the animals were tested in the light-dark box, the reduced latency to enter the lit compartment and the increased time spent there served as indicators for a decrease in the rank order of displayed anxiety: homozygous < heterozygous < wild-type, which is consistent with a gene/dosage effect of the CRH₁ receptor-mediating anxiety-related behavior during alcohol withdrawal (Fig. 21).

10. CRH-binding protein

Further fine-tuning of the HPA system is accomplished by the presence of CRH-binding protein (CRH-BP) (Pot-

ter et al., 1991). This protein binds CRH with high affinity, and as it is localized in the pituitary, it can diminish the production and release of ACTH. CRH-BP is also broadly distributed in the brain, where it colocalizes in some areas with CRH and its receptors, a finding that supports its role as a modulator of CRH-induced behavioral and autonomic effects. In cases where the CRH/CRH-BP ratio is increased, activation of CRH receptor-mediated effects occurs (Potter et al., 1992, 1994). If the amount of CRH but not of CRH-BP is decreased, CRH receptor activation is attenuated. The latter condition is believed to be present in patients with Alzheimer's disease, in whom CRH has been demonstrated to be decreased in cortical areas and cerebrospinal fluid (Bissette et al., 1985; Pomara et al., 1989). In these patients CRH receptors were upregulated and CRH-BP was unchanged (De Souza et al., 1986). A study by Potter et al. (1992) suggested that 60-90% of the total CRH present in the human brain is bound to CRH-BP and thus prevented from exerting biological effects on CRH receptors. It is of note that CRH-BP binds CRH with a 10-fold higher affinity than the CRH₁ and the CRH₂ receptor (Chang et al., 1993; Perrin et al., 1995). The highest concentrations of CRH-BP were found in the hypothalamus, the central amygdala and the hippocampus. The decrease in free CRH and CRH/CRH-BP in patients with Alzheimer's disease was hypothesized to account for the cognitive decline in these patients because CRH was shown to have cognition-enhancing

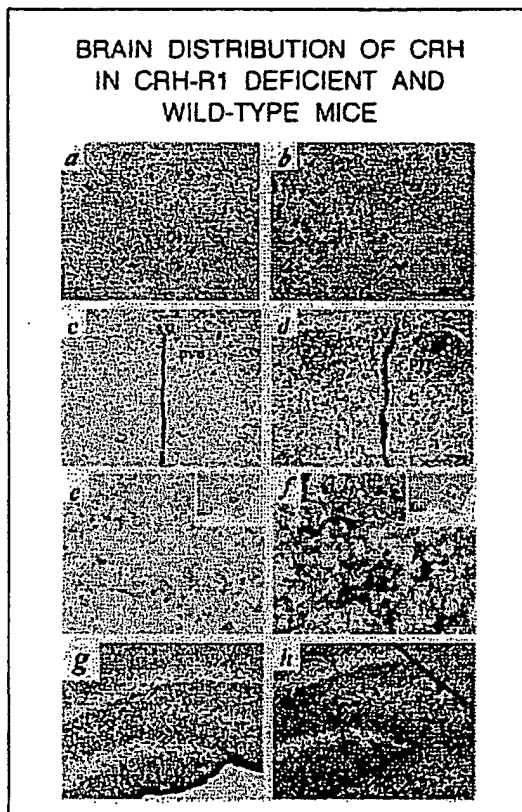


Fig. 19. Immunohistochemical analysis of CRH distribution in wild-type mice (left) and mutants with CRH receptor deficiency (right). In the mutants, CRH is increased in the perikarya and fibers within layer V of the neocortex (a,b), in the paraventricular nucleus (PVN) of the hypothalamus (c,d), in the central amygdala (e,f), and in the polymorphic layer of the hippocampal dentate gyrus (PoDG). These data indicate that via short-loop feedback CRH expression is enhanced in the absence of functional CRH receptors (from: Timpl et al., 1998; A. Kresse and M. Müller, unpublished results).

effects. Consequently, Behan et al. (1995a) administered a peptide (CRH₍₆₋₃₃₎) that displaces CRH from CRH-BP and showed that memory and learning were improved. They also showed that it is possible to dissociate the cognition-enhancing effects of CRH from its anxiogenic arousal effects. Behan et al. (1995a) attributed this to the relative enrichment of CRH-BP in the hippocampus and the cerebral cortex, brain regions that are involved more in cognitive than in emotional responses to CRH. The same group investigated brains of Alzheimer patients and confirmed that CRH-BP is capable of limiting the biological actions of CRH in various brain regions, particularly the cerebral cortex (Behan et al., 1997). Because of the high concentration of CRH in the hippocampus and the stimulatory actions of CRH on hippocampal

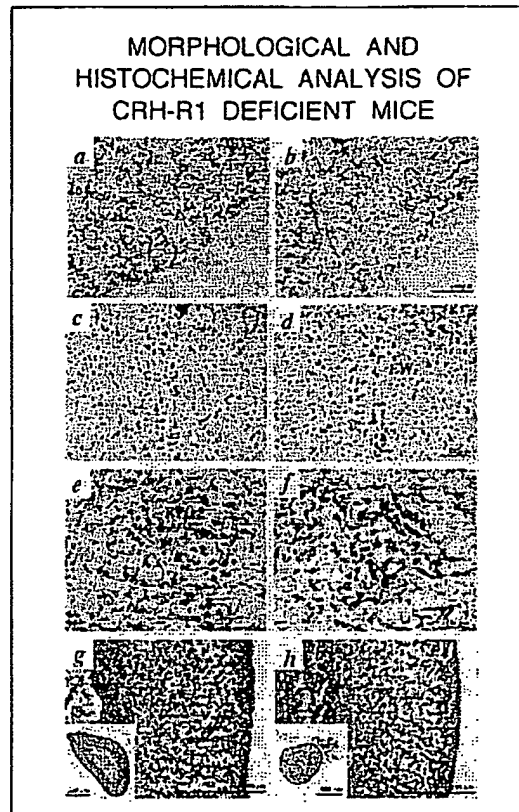


Fig. 20. Morphological and histochemical analysis of CRH receptor-deficient mice: immunohistochemical localization revealed no apparent differences in distribution of corticotrophic cells within the pituitary gland (a = wild-type; b = CRH receptor-deficient mutants). Immunohistochemical localization of urocortin (c = wild-types; d = mutants) is increased in the Edinger-Westphal (EW) nucleus of the mutants. CRH-binding protein is increased in the rostral periolivary (RPO) region. Morphological and histological analysis of the adrenal gland revealed no apparent differences in the size of the subzones of the cortex (r = zona reticularis; f = zona fasciculata; g = zona glomerulosa). Note the marked reduction in the size of the adrenal medulla of mutant mice (adapted from: Timpl et al., 1998 and A. Kresse and M. Müller, unpublished results).

acetylcholine release (Day et al., 1998), it is noteworthy that no significant differences in CRH/CRH-BP or free CRH levels in these brain areas were found between Alzheimer patients and controls.

In the context of investigating increased CRH secretion as a factor in the pathogenesis of depression, drug interventions that lead to increases in CRH-BP would be a worthwhile strategy. Behan et al. (1995b) have pointed out that CRH-BP might exist in various splice forms and that its expression depends on the cell type in the brain. When primary astrocytes are stimulated with forskolin, which directly activates adenyl cyclase to enhance phos-



Fig. 21. The time (latency) until the lit compartment was entered is decreased and the time spent in the lit compartment is increased in CRH₁ receptor-deficient mutants (a). After all mice had been subjected to a forced alcohol-drinking procedure and subsequent withdrawal from alcohol, the anxiety-related behavior (latency and time spent in lit compartment) reflected a gene/dosage effect with the intensity of anxiety increasing with increasing CRH₁ receptor levels (wild-type > heterozygous > homozygous). Similar effects were observed when anxiety-related behavior was assessed by scoring the entries into the lit compartment (b, upper panel) or by counting the animals avoiding the lit compartment (b, lower panel) (adapted from: Timpl et al., 1998).

phorylation of CREB via cAMP and PKA, an elevation of CRH-BP mRNA occurs. The same treatment of neuron and astrocyte cultures elicits CRH-BP levels in the media. This finding would suggest that CRH-BP is regulated by a signaling pathway that is also activated through antidepressants. It is tempting to postulate such a mechanism, but it is important to know that the CRH-BP gene does not contain a CRE, and thus enhanced CRH-BP gene expression through phospho-CREB binding to CRE is unlikely. However, an API site has been identified in the CRH-BP promoter sequence, which may have regulatory function. The API site binds API-related proteins (e.g., *jun*, *fos*), and genes regulated by API have been reported to be negatively modulated by ligand-activated GRs (Schüle et al., 1990). Given the high concentration of CRH-BP in areas where GR levels are also high, it is likely that CRH-BP transactivation through API is repressed by formation of an API-GR complex, preventing API-DNA binding and transactivation of API-regulated genes. If such a mechanism exists in the human brain, elevated corticosteroid levels would prevent complexing abundant CRH through suppressing

API-activated CRH-BP expression and consequently enhancing the behavioral and neuroendocrine effects of CRH. Whether such a mechanism is involved is still uncertain, but the question can be addressed by future research.

11. Psychiatric indications for CRH receptor antagonists

The reason for HPA hyperactivity and, in particular, for enhanced production and release of CRH in depression is not yet known. Genetic and experience-related factors may interact to induce manifold changes in corticosteroid receptor signaling. According to the concept developed by Ronald de Kloet et al. (1998), once the balance of MR- and GR-mediated events is disturbed, an individual loses the ability to maintain homeostasis if challenged, for example, by experiencing an adverse life event. This leads to a condition of disturbed neuroendocrine regulation and impaired behavioral adaptation, which, if a certain threshold is surpassed, may

trigger the onset of a psychiatric disorder to which the individual is prone.

The complex regulation of the HPA system provides multiple levels for intervention. A straightforward way to suppress hyperactivity is to use steroid synthesis inhibitors (Murphy, 1991). This strategy has not yet yielded convincing results. Another approach is to use corticosteroid receptor antagonists, which should be effective only under conditions in which central corticosteroid abundance has negative effects on neurocircuitries involved in the development of psychosis. The enhancement of dopaminergic neurotransmission by elevated cortisol secretion is perhaps responsible for delusions in patients with psychotic depression, whereas other symptoms such as psychomotor changes, loss of appetite, and libido and sleep disturbances are more directly caused by CRH. Consequently, a rapid blockade of central cortisol function might be desirable as a first step. Corticosteroid receptor blockers would be a possibility, but as Reul et al. (1993, 1994b) have shown, functional GRs and MRs are essential for conferring long-term antidepressant efficacy, including attenuation of the HPA response to stress. Therefore, only brief treatment with a GR antagonist seems appropriate. Alternatively, a low dosage of dexamethasone, which lowers corticosteroid receptor occupation by suppressing ACTH and cortisol, would be worth clinical testing in patients with psychotic depression. Due to the decreased ability of low dosages of dexamethasone to enter the brain, the loss of cortisol, the main endogenous ligand, is not compensated in the CNS, resulting in a functional antagonism of central GRs and MRs, which increase in number in response to this condition. The good antidepressant efficacy of trimipramine, especially in patients with psychotic depression, is consistent with the reported effects of cortisol on dopaminergic neurotransmission. Trimipramine suppresses the HPA system most effectively in patients with depression (Holshoer-Trachsler et al., 1994; Sonntag et al., 1996).

In general, antidepressants suppress the HPA system after long-term administration, but this effect takes considerable time and is closely linked to the time when the clinical effects of antidepressant drug treatment become apparent. A promising strategy to shorten the time span until antidepressants act by suppressing CRH gene activation and release is the blockade of CRH receptors. All relevant preclinical experiments show that decreasing CRH₁ receptor function either by blockade or by suppressing its synthesis through CRH₁ receptor gene deletion results in a decrease in anxiety in stressed animals. If the preclinical data available for CRH₁ receptor antagonists and those for benzodiazepines are compared, it is not difficult to predict that CRH₁ receptor antagonists will act as anxiolytics. The major difference between these compounds and benzodiazepines is that the latter have some anxiolytic, sedating or hypnotic effects

under any conditions, whereas CRH₁ receptor antagonists are effective only if CRH is hypersecreted. The CRH₁ receptor knockout mice illustrate this, as they still have normal ACTH levels when unstressed, indicating that blockade of this receptor would not interfere with baseline HPA activity. However, these animals show elevated CRH levels in the central amygdala and hypothalamus, raising the question of whether there might be a rebound after cessation of long-term CRH₁ receptor blockade and whether a combination of CRH₁ receptor antagonists and antidepressants would present an advantageous strategy.

Many clinical conditions are accompanied by an exaggerated stress response. Theoretically, all such conditions are potential indications for the use of CRH receptor antagonists. In addition to anxiety and depression, alcohol withdrawal is another very likely indication. This condition has been shown to be associated with excessive HPA activity in both humans and animals. If rats are treated with CRH antagonists the signs of withdrawal are much less severe. Similarly, mouse mutants lacking functional CRH₁ receptors show less severe signs and symptoms during withdrawal from long-term alcohol exposure than do wild-type mice (Timpl et al., 1998). In general, addictive behavior is reinstated by stress in animals and humans after prolonged drug-free periods. A testable hypothesis is the use of CRH₁ receptor antagonists as a preventive strategy to prolong the period of abstinence. Another possible indication for the use of CRH₁ receptor antagonists is a stress-related sleep disturbance, which is typical in depression and under stress, and which can be induced by administration of CRH in rats and humans (Ehlers et al., 1986; Holshoer et al., 1988). The possibility that CRH₁ receptor antagonists may also be of value in treating the neurological consequences of traumatic brain injury has been raised by the group of Nancy Rothwell in Manchester, U.K. After unilateral permanent occlusion of the middle cerebral artery in rats it was observed that the neuronal damage following the ischemic insult was reduced by α -hCRH₁ when given before and after the trauma (Roe et al., 1998). Similarly, in an earlier study, it was possible to reduce the NMDA receptor agonist-induced excitotoxic neuronal damage by α -hCRH₁ administration (Strijbos et al., 1994). These and subsequent experiments indicate that CRH is involved in the neuronal damage that develops after brain injuries, resulting from trauma or excitotoxic causes. These neuronal alterations can be limited by CRH antagonists (Roe et al., 1998). Translated into clinical conditions, these findings suggest that immediate administration of a CRH receptor antagonist, for example after an ischemic insult, might reduce the volume of damaged brain tissue.

Combining molecular genetics with behavioral pharmacology allowed to identify the CRH₁ receptor as a most prominent target for new drugs. Combinatorial

chemistry and high throughput screens led to the generation of a number of candidate compounds that, after structural modification, fulfil the requirements of a CRH₁ receptor antagonist with a promising pharmacological profile. Some of these compounds that emerged from a straightforward 'from bed-to-bench-and-back' strategy are now under clinical investigation.

References

- Aguilera G. Regulation of pituitary ACTH secretion during chronic stress. *Front Neuroendocrinol* 1994;15:321-50.
- Aldenhoff JB, Gruol DL, Rivier J, Vale W, Siggins GR. Corticotrophin-releasing factor decreases postburst hyperpolarizations and excites hippocampal neurons. *Science* 1983;221:875-7.
- Antoni F. Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of 41-residue CRF. *Endocrine Rev* 1986;7:351-8.
- Arato M, Banki CM, Bissette G, Nemeroff CB. Elevated CSF CRH in suicide victims. *Biol Psychiatry* 1989;25:355-9.
- Baldwin HA, Rasmick S, Koob GF, Britton KT. CRF antagonist reverses the 'anxiogenic' response to ethanol withdrawal in the rat. *Psychopharmacology* 1991;103:227-32.
- Banki CM, Bissette G, Arato M, O'Connor L, Nemeroff CB. Cerebrospinal fluid corticotropin-releasing factor-like immunoreactivity in depression and schizophrenia. *Am J Psychiatry* 1987;144:873-7.
- Baram TZ, Hirsch E, Sneed OC 3rd, Schultz L. Corticotropin-releasing hormone-induced seizures in infant rats originate in the amygdala. *Ann Neurol* 1992;31:488-94.
- Baram TZ, Mitchell WG, Haden E. Inhibition of pituitary-adrenal secretion by a corticotropin releasing hormone antagonist in humans. *Mol Psychiatry* 1996a;1:320-4.
- Baram TZ, Koutsoukos Y, Schultz L, Rivier J. The effect of 'Astressin', a novel antagonist of corticotropin releasing hormone (CRH), on CRH-induced seizures in the infant rat: comparison with two other antagonists. *Mol Psychiatry* 1996b;1:223-6.
- Baram TZ, Chalmers DT, Chen C, Koutsoukos Y, de Souza EB. The CRF1 receptor mediates the excitatory actions of corticotropin releasing factor (CRF) in the developing rat brain: in vivo evidence using a novel, selective, non-peptide CRF receptor antagonist. *Brain Res* 1997;770:89-95.
- Barden N, Stec SM, Montkowski A, Holsboer F, Reul JMHM. Endocrine profile and neuroendocrine challenge test in transgenic mice expressing antisense RNA against the glucocorticoid receptor. *Neuroendocrinology* 1997;66:212-20.
- Behan DP, Heinrichs SC, Troncoso JC, Liu X-J, Kawas CH, Ling N, de Souza EB. Displacement of corticotropin releasing factor from its binding protein as a possible treatment for Alzheimer's disease. *Nature* 1995a;378:284-7.
- Behan DP, Maciejewski D, Chalmers D, de Souza EB. Corticotropin releasing factor binding protein (CRF-BP) is expressed in neuronal and astrocytic cells. *Brain Res* 1995b;698:259-64.
- Behan DP, Khongsaly O, Owens MJ, Chung HD, Nemeroff CB, de Souza EB. Corticotropin-releasing factor (CRF), CRF-binding protein (CRF-BP), and CRF/CRF-BP complex in Alzheimer's disease and control postmortem human brain. *J Neurochem* 1997;68:2052-60.
- Behl C. Alzheimer's disease and oxidative stress: implications for novel therapeutic approaches. *Prog Neurobiol* 1998;56:1-23.
- Bissette G, Reynolds GP, Kils CD, Widerlov W, Nemeroff CB. Corticotropin-releasing factor-like immunoreactivity in senile dementia of the Alzheimer type. *JAMA* 1985;254:3067-9.
- Bliss TVP, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 1993;361:31-9.
- Bourgeois S, Gruol DJ, Newby RF, Rajah FM. Expression of an mdr gene is associated with a new form of resistance to dexamethasone-induced apoptosis. *Mol Endocrinol* 1993;7:840-51.
- Bradbury M, Akana SF, Dallman MF. Roles of type 1 and type 2 corticosteroid receptors in the regulation of basal activity in the hypothalamic-pituitary-adrenal axis during the diurnal trough and peak: evidence for a non-additive effect of combined receptor occupation. *Endocrinology* 1994;134:1286-96.
- Britton DR, Koob GF, River H, Vale W. Intraventricular corticotropin-releasing factor enhances behavioral effects of novelty. *Life Sci* 1982;31:363-7.
- Britton KT, Lee G, Vale W, Rivier J, Koob GF. Corticotropin-releasing factor (CRF) receptor antagonist blocks activating and 'anxiogenic' actions of CRF in the rat. *Brain Res* 1986;369:303-6.
- Butler PD, Weiss JM, Stout JC, Nemeroff CB. Corticotropin-releasing factor produces fear-enhancing and behavioral activating effects following infusion into the locus coeruleus. *J Neurosci* 1990;10:176-83.
- Chen Z, McCaslin PP. Amitriptyline, desipramine, cyproheptadine and carbamazepine, in concentrations used therapeutically, reduce kainic- and N-methyl-D-aspartate-induced intracellular Ca^{2+} levels in neuronal culture. *Eur J Pharmacol* 1992;219:53-7.
- Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, Meaney MJ. Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proc Natl Acad Sci USA* 1998;95:5335-40.
- Chalmers DT, Lovenberg TW, De Souza EB. Localization of novel corticotropin-releasing factor receptor (CRF2) mRNA expression to specific subcortical nuclei in rat brain: Comparison with CRF1 receptor mRNA expression. *J Neurosci* 1995;15:6340-50.
- Chalmers DT, Lovenberg TW, Grigoriadis DE, Behan DP, De Souza EB. Corticotropin-releasing factor receptors: from molecular biology to drug design. *Trends Pharmacol Sci* 1996;17:166-72.
- Chang CP, Pearce RV 2nd, O'Connell S, Rosenfeld MG. Identification of a seven transmembrane helix receptor for corticotropin-releasing factor and sauvagine in mammalian brain. *Neuron* 1993;11:1187-95.
- Charlton BG, Cheetham SC, Horton RW, Katona CLE, Crompton MR, Ferrier IN. Corticotropin-releasing factor immunoreactivity in post-mortem brain from depressed suicides. *J Psychopharmacol* 1988;2:13-8.
- Chen C, Dagnino R, De Souza EB, Grigoriadis DE, Huang CQ, Kim K-L, Liu T, Moran T, Webb TR, Witten JP, Xie YF, McCarthy JR. Design and synthesis of a series of non-peptide high affinity human corticotropin releasing factor-1 receptor antagonists. *J Med Chem* 1996;39:4358-60.
- Chen R, Lewis KA, Perrin MH, Vale WW. Expression cloning of a human corticotropin-releasing factor receptor. *Proc Natl Acad Sci USA* 1993;90:8967-71.
- Choi JJ, Huang G-J, Shafik E, Wu W-H, McArdle JJ. Imipramine's selective suppression of an L-type calcium channel in neurons of murine dorsal root ganglia involves G proteins. *J Pharmacol Exp Ther* 1992;263:49-53.
- Collins S, Caron MG, Lefkowitz RJ. β_2 -adrenergic receptors in hamster smooth muscle cells are transcriptionally regulated by glucocorticoids. *J Biol Chem* 1988;263:9067-70.
- Coplan JD, Andrews MW, Rosenblum LA, Owens MJ, Friedman S, Gorman JM, Nemeroff CB. Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult non-human primates exposed to early-life stressors: implications for the pathophysiology of mood and anxiety disorders. *Proc Natl Acad Sci USA* 1996;93:1619-23.
- Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR, Bertino JR. Multidrug-resistance gene (P-gly-

- coprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc Natl Acad Sci USA* 1989;86:695-8.
- Cosin A, Yassin SA, Hucks D, Forsling ML, Besser GM, Grossman A. Differential effects of neuroexcitatory amino acids on corticotropin-releasing hormone-41 and vasopressin release from rat hypothalamic explants. *Endocrinology* 1992;131:2595-602.
- Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. *Science* 1993;262:689-95.
- Dallman MF. Stress Update. Adaptation of the hypothalamic-pituitary-adrenal axis to chronic stress. *Trends Endocrinol Metab* 1993;4:62-9.
- Dautzenberg FM, Dietrich K, Palchoudhuri MR, Spiess J. Identification of two corticotropin-releasing factor receptors from *Xenopus laevis* with high ligand selectivity: unusual pharmacology of the type 1 receptor. *J Neurochem* 1997;69:1640-9.
- Dautzenberg FM, Wille S, Lohmann R, Spiess J. Mapping of the ligand-selective domain of the *Xenopus laevis* corticotropin-releasing factor receptor 1: Implications for the ligand-binding site. *Proc Natl Acad Sci USA* 1998;95:4941-6.
- Day JC, Koehl M, Deroche V, Le Moal M, Stefania M. Prenatal stress enhances stress- and corticotropin-releasing factor-induced stimulation of hippocampal acetylcholine release in adult rats. *J Neurosci* 1998;18:1886-92.
- De Bellis MD, Gold PW, Geraciotti TD Jr, Listwak SJ, Kling MA. Association of fluoxetine treatment with reductions in CSF concentrations of corticotropin-releasing hormone and arginine vasopressin in patients with major depression. *Am J Psychiatry* 1993;150:656-7.
- De Goeij DC, Kvetnansky R, Whitnall MH, Jezova D, Berkenbosch F, Tilders FJ. Repeated stress-induced activation of corticotropin-releasing factor neurons enhances vasopressin stores and colocalization with corticotropin-releasing factor in the median eminence of rats. *Neuroendocrinology* 1991;53:150-9.
- De Kloet ER, Vreugdenhil E, Oitzl MS, Joels M. Brain corticosteroid receptor balance in health and disease. *Endocrine Rev* 1998;19:269-301.
- De Souza EB, Whitehouse PJ, Kulhar MJ, Price DL, Vale WW. Reciprocal changes in corticotropin-releasing factor (CRF)-like immunoreactivity and CRF receptors in cerebral cortex of Alzheimer's disease. *Nature* 1986;319:593-5.
- Dijkstra I, Tilders FJH, Aguilera G, Kiss A, Rabaud-Diehl C, Barden N, Karanth S, Holsboer F, Reul JMHM. Reduced activity of hypothalamic corticotropin-releasing hormone neurons in transgenic mice with impaired glucocorticoid receptor function. *J Neurosci* 1998;18:3009-18.
- Drouin J, Maira M, Phillips A. Novel mechanism of action for Nur77 and antagonism by glucocorticoids: a convergent mechanism for CRH activation and glucocorticoid repression of POMC gene transcription. *J Steroid Biochem Mol Biol* 1998;65:59-63.
- Duman RS, Nestler EJ. Signal transduction pathways for catecholamine receptors. In: Bloom FE, Kupfer DJ, editors. *Psychopharmacology: The Fourth Generation of Progress*. New York, Raven Press, 1995:303-20.
- Duman RS, Heninger GR, Nestler EJ. A molecular and cellular theory of depression. *Arch Gen Psychiatry* 1997;54:597-606.
- Dunn AJ, File SE. Corticotropin-releasing factor has an anxiogenic action in the social interaction test. *Hormones Behav* 1987;21:193-202.
- Dunn AJ, Berridge CW. Physiological and behavioral response to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? *Brain Res Rev* 1990;15:71-100.
- Ehlers CL, Reed TK, Henriksen SJ. Effects of corticotropin-releasing factor and growth hormone-releasing factor on sleep and activity in rats. *Neuroendocrinology* 1986;42:467-74.
- Gallagher M, Chiba AA. The amygdala and emotion. *Curr Opin Neurobiol* 1996;6:221-7.
- Geraciotti TD Jr, Orth DN, Ekhtator NN, Blumenkopf B, Loosen PT. Serial cerebrospinal fluid corticotropin-releasing hormone concentrations in healthy and depressed humans. *J Clin Endocrinol Metab* 1992;74:1325-30.
- Geraciotti TD Jr, Loosen PT, Orth DN. Low cerebrospinal fluid corticotropin-releasing hormone concentrations in eucortisolemic depression. *Biol Psychiatry* 1997;42:166-74.
- Gold PW, Chrousos GP, Kellner C, Post R, Roy A, Augerinos P, Schulte H, Oldfield E, Loriaux DL. Psychiatric implications of basic and clinical studies with corticotropin-releasing factor. *Am J Psychiatry* 1984;141:619-27.
- Griebel G, Perrault G, Sanger DJ. Characterization of the behavioral profile of the non-peptide CRF receptor antagonist CP-154,526 in anxiety models in rodents. Comparison with diazepam and buspirone. *Psychopharmacology* 1998;138:55-66.
- Hauger RL, Aguilera G. Regulation of pituitary corticotropin-releasing hormone receptors by CRH: interaction with vasopressin. *Endocrinology* 1993;133:1708-14.
- Hauger RL, Irwin MR, Lorang M, Aguilera G, Brown MR. High intracerebral levels of CRH result in CRH receptor downregulation in the amygdala and neuroimmune desensitization. *Brain Res* 1993;616:283-92.
- Hauger RL, Dautzenberg FM, Flaccus A, Liepold T, Spiess J. Regulation of corticotropin-releasing factor receptor function in human Y-79 retinoblastoma cells: rapid and reversible homologous desensitization but prolonged recovery. *J Neurochem* 1997;68:2508-16.
- Heck S, Kullmann M, Gast A, Ponta H, Rahmsdorf HJ, Herrlich P, Cato AC. A distinct modulating domain in glucocorticoid receptor monomers in the repression of activity of the transcription factor AP-1. *EMBO J* 1994;13:4087-95.
- Heinrichs SC, Merlo-Pich E, Miczeck KA, Britton KT, Koob GF. Corticotropin-releasing factor antagonist reduces emotionality in socially defeated rats via direct neurotropic action. *Brain Res* 1992;581:190-7.
- Heinrichs SC, Menzaghi F, Merlo-Pich E, Baldwin HA, Rassnick S, Britton KT, Koob GF. Anti-stress action of a corticotropin-releasing factor antagonist on behavioral reactivity to stressors of varying type and intensity. *Neuropsychopharmacology* 1994;11:179-86.
- Heinrichs SC, Stenzel-Poore MP, Gold LH, Battenberg E, Bloom FE, Koob GF, Vale WW, Merlo-Pich E. Learning impairment in transgenic mice with central overexpression of corticotropin-releasing factor. *Neuroscience* 1996;74:303-11.
- Heinrichs SC, Lapansky J, Lovenberg TW, De Souza EB, Chalmers DT. Corticotropin-releasing factor CRF₁, but not CRF₂, receptors mediate anxiogenic-like behavior. *Regul Peptides* 1997;71:15-21.
- Heuser I, Yassouridis A, Holsboer F. The combined dexamethasone/CRH test: A refined laboratory test for psychiatric disorders. *J Psychiatric Res* 1994;28:341-56.
- Heuser IJE, Schweiger U, Gotthardt U, Schmider J, Lammers CH, Dentling M, Yassouridis A, Holsboer F. Pituitary-adrenal-system regulation and psychopathology during amitriptyline treatment in elderly depressed patients and in normal comparison subjects. *Am J Psychiatry* 1996;153:93-9.
- Heuser I, Deuschle M, Weber B, Stalla GK, Holsboer F. Increased activity of the hypothalamus-pituitary-adrenal system after treatment with the mineralocorticoid receptor antagonist spironolactone. *Psychoneuroendocrinology* 1998; in press.
- Holsboer F. Prediction of clinical course by dexamethasone suppression test (DST) response in depressed patients—physiological and clinical construct validity of the DST. *Pharmacopsychiatry* 1983;16:186-91.
- Holsboer F. Neuroendocrinology of mood disorders. In: Bloom FE, Kupfer DJ, editors. *Psychopharmacology: The Fourth Generation of Progress*. New York, Raven Press, 1995a:957-69.
- Holsboer F, Barden N. Antidepressants and HPA regulation. *Endocrine Rev* 1996;17:187-205.
- Holsboer F, von Bardeleben U, Gerken A, Stalla GK, Müller OA. Blunted corticotropin and normal cortisol response to human cort-

- corticotropin-releasing factor in depression. *N Engl J Med* 1984a;311:1127.
- Holsboer F, Möller OA, Doerr HG, Sippell WG, Stalla GK, Gerken A, Steiger A, Boll E, Benkert O. ACTH and multiteroid responses to corticotropin-releasing factor in depressive illness: relationship to multiteroid responses after ACTH stimulation and dexamethasone suppression. *Psychoneuroendocrinology* 1984b;9:147-69.
- Holsboer F, Gerken A, von Bardeleben U, Grimm W, Beyer H, Möller OA, Stalla GK. Human corticotropin-releasing hormone in depression. *Biol Psychiatry* 1986;21:601-11.
- Holsboer F, von Bardeleben U, Buller R, Heuser I, Steiger A. Stimulation response to corticotropin-releasing hormone (CRH) in patients with depression, alcoholism and panic disorder. *Hormone Metab Res* 1987a;19:80-8.
- Holsboer F, von Bardeleben U, Wiedemann K, Möller OA, Stalla GK. Serial assessment of corticotropin-releasing hormone response after dexamethasone in depression - Implications for pathophysiology of DST nonsuppression. *Biol Psychiatry* 1987b;22:228-34.
- Holsboer F, von Bardeleben U, Steiger A. Effects of intravenous corticotropin-releasing hormone upon sleep-related growth hormone surge and sleep EEG in man. *Neuroendocrinology* 1988;48:32-8.
- Holsboer F, Spengler D, Heuser I. The role of corticotropin-releasing hormone in the pathogenesis of Cushing's disease, anorexia nervosa, alcoholism, affective disorders and dementia. *Prog Brain Res* 1992;93:385-417.
- Holsboer F, Lauer CJ, Schreiber W, Krieg J-C. Altered hypothalamic-pituitary-adrenocortical regulation in healthy subjects at high familial risk for affective disorders. *Neuroendocrinology* 1995b;62:340-7.
- Holsboer-Trachsler E, Henimenter U, Hatzinger M, Seifritz E, Gerhard U, Hobi V. Sleep deprivation and bright light as potential augmenters of antidepressant drug treatment—neurobiological and psychometric assessment of course. *J Psychiatric Res* 1994;28:381-99.
- Hsu DT, Chen F-L, Takahashi LK, Kalin NH. Rapid stress-induced elevations in corticotropin-releasing hormone mRNA in rat central amygdala nucleus and hypothalamic paraventricular nucleus: An *in situ* hybridization analysis. *Brain Res* 1998;788:305-10.
- Hucks D, Lowther S, Crompton MR, Kutona CLE, Horton RW. Corticotropin-releasing factor binding sites in cortex of depressed suicides. *Psychopharmacology* 1997;134:174-8.
- Imaki T, Vale W. Chlordiazepoxide attenuates stress-induced accumulation of corticotropin-releasing factor mRNA in the paraventricular nucleus. *Brain Res* 1993;623:223-8.
- Iredale PA, Terwilliger R, Widnell KL, Nestler EJ, Duman RS. Differential regulation of corticotropin-releasing factor₁ receptor expression by stress and agonist treatments in brain and cultured cells. *Mol Pharmacol* 1996;50:1103-10.
- Iredale PA, Buncley R, Duman RS. Phorbol ester and calcium regulation of corticotropin-releasing factor receptor 1 expression in a neuronal cell line. *J Neurochem* 1997;69:1912-19.
- Jezova D, Oprsalova Z. Adrenocorticotropin release induced by *N*-methyl-D-aspartate or stress: mediation by the area postrema. *J Neuroendocrinol* 1992;4:145-7.
- Joanny P, Steinberg J, Oliver C, Grino M. Glutamate and *N*-methyl-D-aspartate stimulate rat hypothalamic corticotropin-releasing factor secretion *in vitro*. *J Neuroendocrinol* 1997;9:93-7.
- Joshi J, Sabol SL. Proenkephalin gene expression in C6 rat glioma cells: potentiation of cyclic adenosine 3',5'-monophosphate-dependent transcription by glucocorticoids. *Mol Endocrinol* 1991;5:1069-80.
- Kalin NH. Behavioral and endocrine studies of corticotropin-releasing hormone in primates. In: De Souza EB, Nemeroff CB, editors. *Corticotropin-Releasing Factor: Basic and Clinical Studies of a Neuropeptide*. Boca Raton: CRC Press, 1990:275-89.
- Kendler KS. Major depression and the environment: a psychiatric genetic perspective. *Pharmacopsychiatry* 1998;31:5-9.
- Kendler KS, Karkowski-Shuman L. Stressful life events and genetic liability to major depression: genetic control of exposure to the environment? *Psychol Med* 1997;27:539-47.
- Koob GF, Bloom FE. Corticotropin-releasing factor and behavior. *Fed Proc* 1985;44:259-63.
- Koob GF, Heinrichs SC, Merlo-Pich E, Menzaghi F, Baldwin H, Miczek H, Britton KT. The role of corticotropin-releasing factor in behavioural response to stress. In: De Souza EB, Nemeroff CB, editors. *Corticotropin-Releasing Factor: Basic and Clinical Studies of a Neuropeptide*. Boca Raton: CRC Press, 1993:277-95.
- Koob GF, Heinrichs SC, Menzaghi F, Pich EM, Britton KT. Corticotropin-releasing factor, stress and behavior. *Semin Neurosci* 1994;6:221-9.
- Korte SM, Korte-Bouws GAH, Bohus B, Koob GF. Effect of corticotropin-releasing factor antagonist on behavioral and neuroendocrine responses during exposure to defensive burying paradigm in rats. *Physiol Behav* 1994;56:115-20.
- Kostich WA, Chen A, Sperle K, Largent BL. Molecular identification and analysis of a novel human corticotropin-releasing factor (CRF) receptor: the CRF₂ receptor. *Mol Endocrinol* 1998;12:1077-85.
- Kupfer DJ. Management of recurrent depression. *J Clin Psychiatry* 1993;54 Suppl 2:29-33.
- LaBar KS, Gatenby JC, Gore JC, LeDoux JE, Phelps EA. Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. *Neuron* 1998;20:937-45.
- Ladd CO, Owens MJ, Nemeroff CB. Persistent changes in corticotropin-releasing factor neuronal systems induced by maternal deprivation. *Endocrinology* 1996;137:1212-18.
- Landgraf R, Gerstberger R, Montkowski A, Probst JC, Wotjak CT, Holsboer F, Engelmann M. V1 vasopressin receptor antisense oligodeoxynucleotide into septum reduces vasopressin binding, social discrimination abilities and anxiety-related behavior in rats. *J Neurosci* 1995;15:4250-58.
- Lauer CJ, Schreiber W, Modell S, Holsboer F, Krieg J-C. The Munich vulnerability study on affective disorders: overview of the cross-sectional observations at index investigation. *J Psychiatric Res* 1998;32:393-401.
- Leake A, Perry EK, Perry RH, Fairbairn AF, Ferrier IN. Cortical concentrations of corticotropin-releasing hormone and its receptor in Alzheimer type dementia and major depression. *Biol Psychiatry* 1990;28:603-8.
- Leikowitz RJ. G protein-coupled receptor kinase. *Cell* 1993;74:409-12.
- Liebsch G, Landgraf R, Gerstberger R, Probst JC, Wotjak CT, Engelmann M, Holsboer F, Montkowski A. Chronic infusion of a CRH₁ receptor antisense oligodeoxynucleotide into the central nucleus of the amygdala reduced anxiety-related behavior in socially defeated rats. *Regul Peptides* 1995;59:229-39.
- Liebsch G, Montkowski A, Holsboer F, Landgraf R. Behavioural profiles of two Wistar rat lines selectively bred for high or low anxiety-related behaviour. *Behav Brain Res* 1998;94:301-10.
- Liebsch G, Landgraf R, Engelmann M, Lörcher P, Holsboer F. Differential behavioural effects of chronic infusion of CRH₁ and CRH₂ receptor antisense oligonucleotides into the rat brain. *J Psychiatric Res* 1999;33:155-163.
- Linthorst ACE, Flachskamm C, Hopkins SJ, Hoadley ME, Labeur MS, Holsboer F, Reul JMHM. Long-term intracerebroventricular infusion of corticotropin-releasing hormone alters neuroendocrine, neurochemical, autonomic, behavioral, and cytokine responses to a systemic inflammatory challenge. *J Neurosci* 1997;17:4448-60.
- Lisansky J, Peake GT, Strassman RJ, Qualls C, Meikle AW, Risch SC, Fava GA, Zownir-Brazis M, Hochla P, Britton D. Augmented pituitary corticotropin response to a threshold dosage of human corticotropin-releasing hormone in depressives pre-treated with meprobamate. *Arch Gen Psychiatry* 1989;46:641-9.
- Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky PM, Meaney MJ. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science* 1997;277:1659-62.

- Lovenberg TW, Liaw CW, Grigoridis DE, Clevenger W, Chalmers DT, De Souza EB, Oltersdorf T. Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. *Proc Natl Acad Sci USA* 1995;92:836-40.
- Lundkvist J, Chai Z, Tehmian R, Hasanvan H, Bartfai T, Jenck F, Widmer U, Moreau JL. A non-peptidic corticotropin releasing factor receptor antagonist attenuates fever and exhibits anxiolytic-like activity. *Eur J Pharmacol* 1996;304:195-200.
- Luo X, Kiss A, Makara G, Lohit SJ, Aguilera G. Stress-specific regulation of corticotropin releasing hormone receptor expression in the paraventricular and supraoptic nuclei of the hypothalamus in the rat. *J Neuroendocrinol* 1994;6:689-96.
- Malkoski SP, Handanos CM, Dorin RI. Localization of a negative glucocorticoid response element of the human corticotropin releasing hormone gene. *Mol Cell Endocrinol* 1997;127:189-99.
- Mansbach RS, Brooks EN, Chen YL. Antidepressant-like effects of CP-154,526, a selective CRF₁ receptor antagonist. *Eur J Pharmacol* 1997;323:21-6.
- Mecker RB, Greenwood RS, Hayward JN. Glutamate receptors in the rat hypothalamus and pituitary. *Endocrinology* 1994;134:621-9.
- Meijer OC, de Lange ECM, Breimer DD, de Boer AG, Workel JO, de Kloet ER. Penetration of dexamethasone into brain glucocorticoid targets is enhanced in mdrlA P-glycoprotein knockout mice. *Endocrinology* 1998;139:1789-93.
- Melia KR, Duman RS. Involvement of corticotropin-releasing factor in chronic stress regulation of the brain noradrenergic system. *Proc Natl Acad Sci U.S.A.* 1991;88:8382-6.
- Menzaghi F, Howard RL, Heinrichs SC, Valer W, Rivier J, Koob GF. Characterization of a novel and potent corticotropin-releasing factor antagonist in rats. *J Pharmacol Exp Ther* 1994;269:564-72.
- Merchenthaler J. Corticotropin-releasing factor (CRF)-like immunoreactivity in the rat central nervous system. Extrahypothalamic distribution. *Peptides* 1994;5:53-69.
- Meyer TE, Habener JF. Cyclic adenosine 3',5'-monophosphate response element binding protein (CREB) and related transcription-activating deoxyribonucleic acid-binding proteins. *Endocrine Rev* 1993;14:269-90.
- Michelson D, Galliven E, Hill L, Demitrack M, Chrousos G, Gold P. Chronic imipramine is associated with diminished hypothalamic-pituitary-adrenal axis responsiveness in healthy humans. *J Clin Endocrinol Metab* 1997;82:2601-6.
- Mobley PL, Sulser F. Adrenal corticoids regulate sensitivity of nor-adrenaline receptor-coupled adenylate cyclase in brain. *Nature* 1980;286:608-9.
- Modell S, Yassouridis A, Huber J, Holsboer F. Corticosteroid receptor function is decreased in depressed patients. *Neuroendocrinology* 1997;65:216-22.
- Modell S, Lauer CJ, Schreiber W, Huber J, Krieg J-C, Holsboer F. Hormonal response pattern in the combined DEX-CRH test is stable over time in subjects at high familial risk for affective disorders. *Neuropsychopharmacology* 1998;18:253-62.
- Molchan SE, Hill JL, Martinez RA, Lawlor BA, Mellow AM, Rubinow DR, Bissette G, Nemeroff CB, Sunderland T. CSF somatostatin in Alzheimer's disease and major depression: Relationship to hypothalamic-pituitary-adrenal axis and clinical measures. *Psychoneuroendocrinology* 1993;18:509-19.
- Montkowski A, Barden N, Wojcik C, Stee I, Ganster J, Meaney M, Engelmann M, Reul JMHM, Landgraf R, Holsboer F. Long-term antidepressant treatment reduces behavioural deficits in transgenic mice with impaired glucocorticoid receptor function. *J Neuroendocrinol* 1995;7:841-5.
- Moreau JL, Kilpatrick G, Jenck F. Urocortin, a novel neuropeptide with anxiogenic-like properties. *Neuroreport* 1997;8:1697-1701.
- Mori S, Zanardi R, Popoli M, Garbini S, Brunello N, Smeraldi E, Racagni G, Perez J. cAMP-dependent phosphorylation system after short and long-term administration of mifepristone. *J Psychiatric Res* 1998;32:111-5.
- Murphy BEP. Steroids and depression. *J Steroid Biochem Mol Biol* 1991;38:537-59.
- Murphy BEP and Connely OM. Neuroendocrine regulation of the hypothalamic-pituitary-adrenal axis by the nurr1/nur77 subfamily of nuclear receptors. *Mol Endocrinol* 1997;11:39-47.
- Nemeroff CB, Widerlov E, Bissette G, Walleus H, Karlsson I, Eklund K, Kilts DC, Loosen PT, Vale W. Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* 1984;226:1342-4.
- Nemeroff CB, Owens MJ, Bissette G, Andorn AC, Stanley M. Reduced corticotropin releasing factor binding sites in the frontal cortex of suicide victims. *Arch Gen Psychiatry* 1988;45:577-9.
- Nestler EJ, Terwilliger RZ, Duman RS. Chronic antidepressant administration alters the subcellular distribution of cyclic AMP-dependent protein kinase in rat frontal cortex. *J Neurochem* 1989;53:1644-7.
- Nibuya M, Nestler EJ, Duman RS. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *J Neurosci* 1996;16:2365-72.
- Orth DN. Corticotropin-releasing hormone in humans. *Endocrine Rev* 1992;13:164-91.
- Owens MJ, Nemeroff CB. The physiology and pharmacology of corticotropin-releasing factor. *Pharmacol Rev* 1992;43:425-73.
- Owens MJ, Bissette G, Nemeroff CB. Acute effects of alprazolam and adimazolam on the concentration of corticotropin-releasing factor in the rat brain. *Synapse* 1989;4:196-202.
- Parkes D, Rivest S, Lee S, Rivier C, Vale W. Corticotropin-releasing factor activates c-fos, NGF-B, and corticotropin-releasing factor gene expression within the paraventricular nucleus of the rat hypothalamus. *Mol Endocrinol* 1993;7:1357-67.
- Patchev VK, Shoaib M, Holsboer F, Almeida OFX. The neurosteroid tetrahydroprogesterone counteracts CRH-induced anxiety and alters the release and gene expression of CRH in the rat hypothalamus. *Neuroscience* 1994a;62:265-71.
- Patchev VK, Karalis K, Chrousos GP. Effects of excitatory amino acid transmitters on hypothalamic corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) release in vitro: implications in pituitary-adrenal regulation. *Brain Res* 1994b;633:312-6.
- Patchev VK, Montkowski A, Rouskov D, Koranyi L, Holsboer F, Almeida OFX. Neonatal treatment of rats with the neurosteroid tetrahydrodeoxycorticosterone (THDOC) abolishes the behavioral and neuroendocrine consequences of adverse early life events. *J Clin Invest* 1997;99:962-6.
- Paul SM, Purdy RH. Neuroactive steroids. *FASEB J* 1992;6:2311-22.
- Pepin MC, Beaulieu S, Barden N. Antidepressants regulate glucocorticoid receptor messenger RNA concentrations in primary neuronal cultures. *Mol Brain Res* 1989;6:77-83.
- Pepin MC, Pothier F, Burden N. Impaired type II glucocorticoid-receptor function in mice bearing antisense RNA transgene. *Nature* 1992;355:725-8.
- Perez J, Tinelli D, Brunello N, Racagni G. cAMP-dependent phosphorylation of soluble and crude microtubule fractions of rat cerebral cortex after prolonged desmethylimipramine treatment. *Eur J Pharmacol* 1989;172:305-16.
- Perez J, Tinelli D, Bianchi E, Brunello N, Racagni G. cAMP binding proteins in the rat cerebral cortex after administration of selective 5-HT and NE reuptake blockers with antidepressant activity. *Neuropsychopharmacology* 1991;4:57-64.
- Perrin MH, Donaldson CJ, Chen R, Lewis KA, Vale WW. Cloning and functional expression of a rat brain corticotropin releasing factor (CRF) receptor. *Endocrinology* 1993;133:3058-61.
- Perrin M, Donaldson C, Chen R, Blount A, Berggren T, Bilezikian L, Sawchenko P, Vale W. Identification of a second corticotropin-releasing factor receptor gene and characterization of a cDNA expressed in heart. *Proc Natl Acad Sci USA* 1995;92:2969-73.
- Phi Van L, Spengler D, Holsboer F. Glucocorticoid repression of cAMP-dependent hCRH gene promoter activity in a transfected mouse anterior pituitary cell line. *Endocrinology* 1990;127:1412-8.

- Pitts AF, Samuelson SD, Meller WH, Bissette G, Nemeroff CB, Kathos RG. Cerebrospinal fluid corticotropin-releasing hormone, vasopressin, and oxytocin concentrations in treated patients with major depression and controls. *Biol Psychiatry* 1995;38:330-5.
- Plotsky PM. Noli disturbare circulos meos: Integrative role for CRF in organization of the stress response. In: Nappi G, et al. editors. *Stress and the Aging Brain*. New York: Raven Press, 1990.
- Pomara N, Singh RR, Deptula D, Lewitt PA, Bissette G, Stanley M, Nemeroff CB. CSF corticotropin-releasing factor (CRF) in Alzheimer's disease: its relationship to severity of dementia and monoamine metabolites. *Biol Psychiatry* 1989;26:500-4.
- Potter E, Behan DP, Fischer WH, Linton EA, Lowry PJ, Vale WW. Cloning and characterization of the cDNAs for human and rat corticotropin releasing factor-binding proteins. *Nature* 1991;349:423-6.
- Potter E, Behan DP, Linton EA, Lowry PJ, Sawchenko PE, Vale WW. The central distribution of a corticotropin-releasing factor(CRF)-binding protein predicts multiple sites and modes of interaction with CRF. *Proc Natl Acad Sci USA* 1992;89:4192-6.
- Potter E, Sutton S, Donaldson C, Chen R, Perrin M, Lewis K, Sawchenko PE, Vale W. Distribution of corticotropin-releasing factor receptor mRNA expression in the rat brain and pituitary. *Proc Natl Acad Sci USA* 1994;91:8777-81.
- Price ML, Curtis AL, Kirby LG, Valentino RJ, Lucki I. Effects of corticotropin-releasing factor on brain serotonergic activity. *Neuropsychopharmacology* 1998;18:492-502.
- Purba JS, Hoogendijk WJG, Hofman MA, Swaab DF. Increased number of vasopressin- and oxytocin-expressing neurons in the paraventricular nucleus of the hypothalamus in depression. *Arch Gen Psychiatry* 1996;53:137-43.
- Raadshere FC, Hoogendijk WJG, Sturm FC, Tilders FJH, Swaab DF. Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. *Neuroendocrinology* 1994;60:433-6.
- Rabadan-Diehl C, Makara G, Kiss A, Zelena D, Aguilera G. Regulation of pituitary corticotropin-releasing hormone (CRH) receptor mRNA and CRH binding during adrenalectomy: role of glucocorticoids and hypothalamic factors. *J Neuroendocrinol* 1997;9:689-97.
- Racagni G, Brunello N, Tinelli D, Perez J. New biochemical hypotheses on the mechanism of action of antidepressant drugs: cAMP-dependent phosphorylation system. *Pharmacopsychiatry* 1992;25:51-5.
- Rainnie DG, Fernhout BJ, Shinnick-Gallagher P. Differential actions of corticotropin-releasing factor on basolateral and central amygdaloid neurons in vitro. *J Pharmacol Exp Ther* 1992;263:846-58.
- Rangarajan PN, Umesono K, Evans RM. Modulation of glucocorticoid receptor function by protein kinase A. *Mol Endocrinol* 1992;6:1451-7.
- Rassnick S, Heinrichs SC, Britton KT, Koob GF. Microinjection of a corticotropin-releasing factor antagonist into the central nucleus of the amygdala reverses anxiogenic-like effects of ethanol withdrawal. *Brain Res* 1993;605:25-32.
- Reichardt HM, Kuestner KH, Tuckermann J, Kretz O, Wessely O, Bock R, Guss P, Schmid W, Herrlich P, Angel P, Schütz G. DNA binding of the glucocorticoid receptor is not essential for survival. *Cell* 1998;93:531-41.
- Reul JMHM, Stee I, Soder M, Holsboer F. Chronic treatment of rats with the antidepressant amitriptyline attenuates the activity of the hypothalamic-pituitary-adrenocortical system. *Endocrinology* 1993;133:312-20.
- Reul JMHM, Stee I, Wieggers GJ, Labeur MS, Linthorst ACE, Arzt E, Holsboer F. Prenatal immune challenge alters the hypothalamic-pituitary-adrenocortical axis in adult rats. *J Clin Invest* 1994a;93:2600-7.
- Reul JMHM, Labeur MS, Grigoriadis DE, De Souza EB, Holsboer F. Hypothalamic-pituitary-adrenocortical axis changes in the rat after long-term treatment with the reversible monoamine oxidase-A inhibitor moclobemide. *Neuroendocrinology* 1994b;60:509-19.
- Reyes A, Luckhaus J, Ferin M. Unexpected inhibitory action of N-methyl-D,L-aspartate on luteinizing hormone release in adult ovariectomized rhesus monkeys: a role of the hypothalamic-adrenal axis. *Endocrinology* 1990;127:724-9.
- Richards JG, Schoch P, Haring P, Takacs B, Mohler H. Resolving GABA_A/benzodiazepine receptors: cellular and subcellular localization in the CNS with monoclonal antibodies. *J Neurosci* 1987;7:1866-86.
- Rivest S, Laflamme N, Nappi RE. Immune challenge and immobilization stress induce transcription of the gene encoding the CRF receptor in selective nuclei of the rat hypothalamus. *J Neurosci* 1995;15:2680-95.
- Rivier J, Rivier C, Vale W. Synthetic competitive antagonists of corticotropin-releasing factor: effect of ACTH secretion in the rat. *Science* 1984;224:889-91.
- Rodriguez de Fonseca F, Rubio P, Menzaghi F, Merlo-Pich E, Rivier J, Koob GF, Navarro M. Corticotropin-releasing factor (CRF) antagonist [D-Phe¹², Nle²¹⁻²⁴, C-MeLeu²⁷]CRF attenuates the acute actions of the highly potent cannabinoid receptor agonist HU-210 on defensive-withdrawal behavior in rats. *J Pharmacol Exp Ther* 1996;276:56-64.
- Rodriguez de Fonseca F, Carrera MR, Navarro M, Koob GF, Weiss F. Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. *Science* 1997;276:2050-4.
- Roe SY, McGowan EM, Rothwell NJ. Evidence for the involvement of corticotropin-releasing hormone in the pathogenesis of traumatic brain injury. *Eur J Neurosci* 1998;10:553-9.
- Rowe W, Sieverman A, Walker M, Sharma S, Barden N, Seckl JR, Meaney MJ. Antidepressants restore hypothalamic-pituitary-adrenal feedback function in aged, cognitively-impaired rats. *Neurobiol Aging* 1997;18:527-33.
- Roy-Byrne PP, Uhde TW, Post RM, Gallucci W, Chrousos GP, Gold PW. The corticotropin-releasing hormone stimulation test in patients with panic disorder. *Am J Psychiatry* 1986;143:896-9.
- Rueter LE, Jacobs BL. A microdialysis examination of serotonin release in the rat forebrain induced by behavioral/environmental manipulations. *Brain Res* 1996;739:57-69.
- Rupprecht R. The neuropsychopharmacological potential of neuroactive steroids: Cellular and systemic effects. *J Psychiatric Res* 1997;31:297-314.
- Sakane M, Saito N, Taniguchi H, Baba S, Tanaka C. Immunohistochemical localization of γ -aminobutyric acid in the rat pituitary gland and related hypothalamic regions. *Brain Res* 1988;446:343-53.
- Schinkel AH, Wagenaar E, Van Deemter L, Mol CAAM, Borst P. Absence of the mdrla P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *J Clin Invest* 1995;96:1698-705.
- Schüls R, Rangarajan P, Klierer S, Ransone LJ, Bolado J, Yang N, Verma IM, Evans RM. Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor. *Cell* 1990;62:1217-26.
- Schulz DW, Mansbach RS, Sprouse J, Braselton JP, Collins J, Corman M, Dunaiskis A, Faraci S, Schmidt AW, Seeger T, Seymour P, Tingley FD 3rd, Winston EN, Chen YL, Heym J. CP-154,526: A potent and selective nonpeptide antagonist of corticotropin releasing factor receptors. *Proc Natl Acad Sci USA* 1996;93:10477-82.
- Seasholtz AF, Thompson RC, Douglass JO. Identification of a cyclic adenosine monophosphate-responsive element in the rat corticotropin-releasing hormone gene. *Mol Endocrinol* 1988;2:1311-9.
- Shaham Y, Erb S, Leung S, Buczek Y, Stewart J. CP-154,526, a selective, non-peptide antagonist of the corticotropin-releasing factor, receptor attenuates stress-induced relapse to drug seeking in cocaine- and heroin-trained rats. *Psychopharmacology* 1998;137:184-90.

- Sheng M, McFadden G, Greenberg ME. Membrane depolarization and calcium induce *c-fos* transcription via phosphorylation of transcription factor CREB. *Neuron* 1990;4:571–82.
- Sherman JE, Kulin NH. ICV-CRH alters stress-induced freezing behavior without affecting pain sensitivity. *Pharmacol Biochem Behav* 1988;30:801–7.
- Singh VB, Hao-Phan T, Corley KC, Boodle-Biber MC. Increase in cortical and midbrain tryptophan hydroxylase activity by intracerebroventricular administration of corticotropin-releasing factor. Block by adrenalectomy, by RU 38486 and by bilateral lesions to the central nucleus of the amygdala. *Neurochem Int* 1991;20:81–92.
- Sirinathsinghji DJS. Regulation of lordosis behaviour in the female rat by corticotropin-releasing factor, β -endorphin/corticotropin and luteinizing hormone-releasing hormone neuronal systems in the medial preoptic area. *Brain Res* 1986;375:49–56.
- Skutella T, Probst JC, Criswell H, Mory C, Breese G, Jirikowski GF, Holsboer F. Antisense oligodeoxynucleotide complementary to corticotropin-releasing hormone mRNA reduces anxiety in shuttle-box performance. *Neuroreport* 1994a;5:2181–5.
- Skutella T, Montkowski A, Stöhr T, Probst JC, Landgraf R, Holsboer F, Jirikowski GF. Corticotropin-releasing hormone (CRH) antisense oligodeoxynucleotide treatment attenuates social defeat-induced anxiety in rats. *Cell Mol Neurobiol* 1994b;14:579–88.
- Skutella T, Probst JC, Renner R, Holsboer F, Behl C. Corticotropin-releasing hormone receptor (type I) antisense targeting reduces anxiety. *Neuroscience* 1998;85:795–805.
- Smith GW, Aubry J-M, Dellu F, Contarino A, Bilezikjian LM, Gold LH, Hauser C, Bentley CA, Sawchenko PE, Koob GF, Vale W, Lee K-F. Corticotropin-releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. *Neuron* 1998;20:1093–102.
- Sonntag A, Rothe B, Guldner J, Yassouridis A, Holsboer F, Steiger A. Trimipramine and imipramine exert different effects on the sleep EEG and on nocturnal hormone secretion during treatment of major depression. *Depression* 1996;4:1–13.
- Spengler D, Rupprecht R, Phi Van L, Holsboer F. Identification and characterization of a 3',5'-cyclic adenosine monophosphate-responsive element in the human corticotropin-releasing hormone gene promoter. *Mol Endocrinol* 1992;6:1931–41.
- Spina M, Merto-Pich E, Chan RKW, Basso AM, Rivier J, Vale WW, Koob GF. Appetite-suppressing effects of urocortin, a CRF-related neuropeptide. *Science* 1996;273:1561–4.
- Steiger A, Holsboer F. Neuropeptides and human sleep. *Sleep* 1997;20:1038–52.
- Stenzel-Poore R, Yeung W, Cone RD, Rittenberg MB, Stenzel-Poore MP. Identification of a novel murine receptor for corticotropin-releasing hormone expressed in the heart. *Mol Endocrinol* 1995;9:637–45.
- Stenzel-Poore MP, Cameron VA, Vliughan J, Sawchenko PE, Vale W. Development of Cushing's syndrome in corticotropin-releasing factor transgenic mice. *Endocrinology* 1992;130:3378–86.
- Stenzel-Poore MP, Heinrichs SC, Rivest S, Koob GF, Vale WW. Overproduction of corticotropin-releasing factor in transgenic mice: a genetic model of anxiogenic behavior. *J Neurosci* 1994;14:2579–84.
- Strijbos PJLM, Relson JK, Rothwell NJ. Corticotropin-releasing factor antagonist inhibits neuronal damage induced by focal cerebral ischaemia or activation of NMDA receptors in the rat brain. *Brain Res* 1994;656:405–8.
- Sulser F, Vetulani J, Mobley PL. Mode of action of antidepressant drugs. *Biochem Pharmacol* 1978;27:257–71.
- Sutton RE, Koob GF, Le Moal M, Rivier J, Vale W. Corticotropin releasing factor produces behavioural activation in rats. *Nature* 1982;297:331–3.
- Swanson LW, Simmons DM. Differential steroid hormone and neural influences on peptide mRNA levels in CRH cells of the paraventricular nucleus: a hybridization histochemical study in the rat. *J Comp Neurol* 1989;285:413–35.
- Swanson LW, Sawchenko PE, Rivier J, Vale WW. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. *Neuroendocrinology* 1983;36:165–86.
- Swerdlow NR, Britton KT, Koob GF. Potentiation of acoustic startle by corticotropin-releasing factor (CRF) and by fear are both reversed by α -helical CRF (9–41). *Neuropsychopharmacology* 1989;36:165–86.
- Swiergiel AH, Takahashi LK, Kalin NH. Attenuation of stress-induced behavior by antagonism of corticotropin-releasing factor receptors in the central amygdala in the rat. *Brain Res* 1993;623:229–34.
- Takuma K, Matsuda T, Yoshikawa T, Kitanaka J, Gotoh M, Hayata K, Baba A. Corticotropin-releasing factor stimulates Ca^{2+} influx in cultured rat astrocytes. *Biochem Biophys Res Commun* 1994;1103:7.
- Timpl P, Spanagel R, Sillaber I, Kresse A, Reul JMHM, Stalla GK, Blanquet V, Steckler T, Holsboer F, Wurst W. Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. *Nature Genet* 1998;19:162–6.
- Tsai GE, Rugan P, Chang R, Chen S, Linnoila ML, Coyle JT. Increased glutamatergic neurotransmission and oxidative stress after alcohol withdrawal. *Am J Psychiatry* 1998;155:726–32.
- Valdenaire O, Giller T, Breu V, Gottowik J, Kilpatrick G. A new functional isoform of the human CRF₂ receptor for corticotropin-releasing factor. *Biochem Biophys Acta* 1997;1352:129–32.
- Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β -endorphin. *Science* 1981;213:1394–7.
- Valentino RJ, Wehby RG. Corticotropin-releasing factor: evidence for a neurotransmitter role in the locus coeruleus during hemodynamic stress. *Neuroendocrinology* 1988;48:674–7.
- Valentino RJ, Foote SL, Aston-Jones G. Corticotropin-releasing hormone activates noradrenergic neurons of the locus coeruleus. *Brain Res* 1983;270:363–7.
- Valentino RJ, Foote SL, Puge ME. The locus coeruleus as a site for integrating corticotropin-releasing factor and noradrenergic mediation of stress responses. *Ann NY Acad Sci* 1993;697:173–88.
- Van Bockstaele EJ, Colago EEO, Valentino RJ. Corticotropin-releasing factor-containing axon terminals synapse onto catecholamine dendrites and may presynaptically modulate other afferents in the rostral pole of the nucleus locus coeruleus in the rat brain. *J Comp Neurol* 1996;364:523–34.
- Vaughan J, Donaldson C, Bittencourt J, Perrin MH, Lewis K, Sutton S, Chan R, Turnbull AV, Lovejoy D, Rivier C, Rivier J, Sawchenko PE, Vale WW. Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. *Nature* 1995;378:287–92.
- Vita N, Laurent P, Lefort S, Chalon P, Lelias MJ, Kaghad M, Le Fur G, Caput D, Ferrara P. Primary structure and functional expression of mouse pituitary and human brain corticotrophin releasing factor receptors. *FEBS Lett* 1993;335:1–5.
- von Bardeleben U, Holsboer F. Cortisol response to a combined dexamethasone-hCRH challenge in patients with depression. *J Neuroendocrinol* 1989;1:485–8.
- von Bardeleben U, Holsboer F. Effect of age upon the cortisol response to human CRH in depressed patients pretreated with dexamethasone. *Biol Psychiatry* 1991;29:1042–50.
- von Bardeleben U, Holsboer F, Stalla GK, Müller OA. Combined administration of human corticotropin-releasing factor and lysine vasopressin induces cortisol escape from dexamethasone suppression in healthy subjects. *Life Sci* 1985;37:1613–18.
- von Bardeleben U, Stalla GK, Müller OA, Holsboer F. Blunting of ACTH response to human CRH in depressed patients is avoided by metyrapone pre-treatment. *Biol Psychiatry* 1988;24:782–6.
- von Bardeleben U, Heuser I, Holsboer F. Human CRH stimulation response during acute withdrawal and after medium-term absten-

- tion from alcohol abuse. *Psychoneuroendocrinology* 1989;14:441-9.
- Wang L, Martinez V, Vale WW, Tache Y. Peripheral injection of corticotropin-releasing factor (CRF) and a CRF-related peptide, urocortin, activate specific brain areas in rats. *Gastroenterology* 1996;110:A1131.
- Webster EL, Lewis DB, Torpy DJ, Zachman EK, Rice KC, Chrousos GP. In vivo and in vitro characterization of antalarmin, a non-peptide corticotropin-releasing hormone (CRH) receptor antagonist: suppression of pituitary ACTH release and peripheral inflammation. *Endocrinology* 1996;137:5747-50.
- Wiedemann K, Holshoer F. The effect of repeated human corticotropin-releasing hormone administration on dexamethasone-suppressed pituitary-adrenocortical activity in healthy subjects. *Biol Psychiatry* 1997;42:882-8.
- Wiersma A, Baauw AD, Bohus B, Kooblaas JM. Behavioural activation produced by CRH but not α -helical CRH (CRH-receptor antagonist) when microinfused into the central nucleus of the amygdala under stress-free conditions. *Psychoneuroendocrinology* 1995;20:423-32.
- Wilner P. The validity of animal models of depression. *Psychopharmacology* 1984;83:1.
- Wilson MA, Biscardi R, Smith MD, Wilson SP. Effects of benzodiazepine agonist exposure on corticotropin-releasing factor content and hormonal stress responses: divergent responses in male and ovariectomized female rats. *J Pharmacol Exp Ther* 1996;278:1073-82.
- Young EA, Akil H, Haskett RF, Watson SJ. Evidence against changes in corticotroph CRF receptors in depressed patients. *Biol Psychiatry* 1995;37:355-63.
- Zobel A, Yassouridis A, Frieboes R-M, Holshoer F. Cortisol response to the combined dexamethasone-CRH test predicts medium-term outcome in patients with remitted depression. *Am J Psychiatry* 1999; in press.

ENP 00054

CSF corticotropin-releasing hormone and somatostatin in major depression: response to antidepressant treatment and relapse

Csaba M. Banki^a, Lajos Karmacs^a, Garth Bissette^b and Charles B. Nemeroff^c

^aRegional Neuropsychiatric Institute, Nagykallo, Hungary; ^bDuke University Medical Center, Department of Psychiatry, Durham, NC, USA; ^cEmory University School of Medicine, Department of Psychiatry, Atlanta, GA, USA

(Received 28 October, 1991)

(Accepted 22 January, 1992)

Key words: Corticotropin-releasing hormone; Somatostatin; Major depression; Cerebrospinal fluid; Relapse

Summary

Immunoreactive corticotropin-releasing hormone (CRH) and somatostatin (SRIF) were measured in the cerebrospinal fluid (CSF) of 24 female in-patients, suffering from DSM-III-R major depression, both before and after antidepressant treatment. In the total group there were no significant differences between pre- and post-treatment CSF-CRH and SRIF concentrations despite satisfactory clinical improvement in each patient. However, there was a significant post-treatment reduction of the CSF-CRH concentration in the 15 patients who remained depression-free for at least 6 months following treatment, in contrast to the tendency for elevation in those 9 subjects who relapsed within 6 months. CSF-SRIF showed no similar pattern. High, or even increasing, CSF-CRH concentration during antidepressant treatment may indicate lack of normalization of an underlying process in major depression despite symptomatic improvement and predicted early relapse.

Introduction

Neuropeptides have been thought to be involved in the biological pathogenesis of depressive disorders since their role as neurotransmitters, co-transmitters, or neuromodulators, has been recognized. Corticotropin-releasing hormone (CRH), a 41-amino acid peptide regulates corticotropin (ACTH) release from the pituitary and is also a putative neurotransmitter in various brain regions involved in the regulation of affective states and behavioral responses (Nemeroff, 1988; Koob and Bloom, 1985). Central CRH overproduction is one possible mechanism underlying the hypothalamic-pituitary-adrenal (HPA) hyperactivity seen in

many patients with major depression (Gold et al., 1984; Gold and Chrousos, 1985; Holsboer, 1988). In fact, elevated cerebrospinal fluid CRH concentrations were found in Swedish (Nemeroff et al., 1984), American (Bissette et al., 1985) and Hungarian (Banki et al., 1987) patients diagnosed as having major depression, although not all studies agreed (Roy et al., 1987). In one study, reduced CRH binding site number was also reported in the frontal cortex of 26 suicide victims as compared with 29 controls (Nemeroff et al., 1988). More recently a significant decrease of CSF-CRH concentration in nine depressed patients after receiving electroconvulsive treatment was reported (Nemeroff et al., 1991). Synthetic CRH elicits blunted ACTH response in many patients with major depression and the response usually normalizes with clinical recovery (Gold et al., 1984;

Correspondence to: Csaba M. Banki, MD, PhD, P.O. Box 37, H-4321 Nagykallo, Hungary. Tel.: (36-42) 63-133.

Holsboer, 1988). These data, concordant with earlier observations on other measures of HPA hyperactivity in depression, such as plasma or urinary cortisol and the dexamethasone suppression test (Halbreich et al., 1985; Carroll, 1985) suggest that central CRH overproduction may be a state-dependent phenomenon in at least a subgroup of patients with major depression. There are, however, very limited data on CRH changes during antidepressant drug treatment and on its possible predictive value for depressive relapse.

Somatostatin (somatotropin release-inhibiting factor = SRIF) is another neuropeptide widely distributed in the brain and probably acting as a neurotransmitter or a co-transmitter (Nemeroff et al., 1987). There is clinical evidence that SRIF concentrations in the CSF of depressed patients may be significantly reduced during the depressive phase and normalize after recovery (Rubinow et al., 1984; Ågren and Lundqvist, 1984; Post et al., 1988). However, SRIF reduction is not specific to depression but may occur in several neurological and psychiatric disorders (Loosen and Banki, 1988) where it may quite generally indicate the 'active' or 'acute' phases of the disease. HPA hyperactivity (dexamethasone nonsuppression) was found to be associated with low CSF-SRIF in both depressed and schizophrenic subjects (Doran et al., 1986), again suggesting that low CSF-SRIF was also a state-dependent neuropeptide marker. However, electroconvulsive treatment (ECT) caused only a weak, nonsignificant SRIF elevation in depressed patients (Nemeroff et al., 1991). The interrelationships between central SRIF-containing neurone systems and other neurotransmitters (catecholamines, GABA, HPA axis, etc.) and the observation that carbamazepine decreased CSF-SRIF concentrations (Post et al., 1988) make this neuropeptide an important putative mediator of at least some symptoms of depressive disorders. Data on the possible relationship of CSF-SRIF to clinical recovery and relapse have still remained scarce, however.

The present study was performed in severely ill, mostly psychotic depressed inpatients in order to demonstrate CSF neuropeptide changes after successful anti-depressant treatment. In addition, a 6-month follow-up was included to see if either baseline or treatment-induced neuropeptide changes can predict early relapse in major depression.

Patients and methods

Twenty-four female psychiatric in-patients, recently hospitalized in a regional Hungarian hospital specialized in psychiatry, were asked to participate in the study. Their physical and demographic characteristics are summarized in Table 1. All were diagnosed according to the DSM-III-R as having severe major depression; 15 had melancholic features, another 3 had psychotic features, and six had the global severity rating as 'severe' in the DSM-III-R. The large majority of the group had never had manic or hypomanic episodes in the past with the exception of 2 subjects (one bipolar-I and one bipolar-II, in RDC terms). They all gave written consent to participate, after a detailed explanation of the procedure, in accordance with the Helsinki declaration requirements. Pre-treatment examination included a detailed physical, neurological, and laboratory examination to rule out any significant medical illness, endocrine or neurological abnormality; in addition, we excluded individuals with alcohol or other psychoactive substance dependence, pregnancy or lactation, use of steroid drugs within 6 months, and other major psychiatric disorders such as dementia, mental retardation, or personality disorder. None of the patients reported use of antidepressants, neuroleptics, lithium, or anticonvulsant drugs within the last 2 weeks before admission.

After 2–5 days of initial evaluation and stabilization in the hospital environment, and following the obtaining of the written consent in the presence of two staff members, lumbar punctures were performed at 9:00–10:00 a.m. in a sitting position at the fourth intervertebral space. Ten ml CSF was obtained in a plastic tube and

TABLE 1
PHYSICAL AND BACKGROUND VARIABLES IN DEPRESSED PATIENTS

Values are expressed as mean \pm SD (range) where appropriate.

Age (years)	51.4 \pm 10.4	(23–70)
Weight (kg)	63.5 \pm 11.4	(39–80)
Height (cm)	154.9 \pm 6.6	(146–174)
No. episodes	2.5 \pm 1.4	(1–6) not including index episode
First onset (years)	6.2 \pm 7.5	(0.5–30) median 4 years
Episode duration (weeks)	5.3 \pm 3.7	(2–10) median 3 weeks
G.A.F. score (DSM-III-R)	33.0 \pm 10.0	(18–50)
HAMD score	37.1 \pm 5.3	(24–46)
Melancholic type	15/24 patients	

immediately frozen at -70°C without any additional conservant. Before the LP patients received only 1–2 mg alprazolam or 300–600 mg chlormethiazole when necessary to control excessive anxiety or agitation or to promote sleep; LPs were performed after 12 h fasting and an overnight controlled bedrest. No major LP-related complaints were seen following the procedure apart from mild, transient headache in 5 subjects.

Antidepressant drug treatment was initiated after the LP according to routine hospital procedures. The principal drug was 125–225 mg maprotiline ($n = 15$), 480–720 mg dibenzepine ($n = 5$), 150–200 mg amitriptyline ($n = 4$); as adjuvants anxiolytics, thioxanthene neuroleptics were also used. The severity of depression was evaluated on the 24-point Hamilton Depression Scale (HAMD), and the Clinical Global Impression (CGI) scale was also used to measure severity and treatment change. In two patients no significant antidepressant response was achieved after 4 weeks and therefore they received a course of 4 and 6 ECT each (in methohexital anesthesia with succinylcholine relaxation, assisted respiration with oxygen, and cuff monitoring). All 24 patients improved significantly by the 5th to 8th week of treatment as indicated by at least 50% decrease of the HAMD score and at least 'much improved' on the CGI. Final HAMD scores were between 6 and 22, still indicating clinically significant depressive symptomatology in some patients ($n = 5$) while 19 others had no final HAMD rating above 14.

A second LP was performed in the 6th to 8th week of treatment, before discharge, when clinically significant improvement occurred; the procedure was the same as with the first LP but antidepressant drug treatment was not interrupted. All subjects remained under hospital supervision for at least 24 h following the LP.

All CSF samples were stored at -70°C in darkness for 1–3 months and then transported by air in a plastic container containing dry ice to the United States (Dr. Nemeroff, Psychoendocrine Laboratory, Duke University Medical Center) for peptide analysis. All samples were coded before transportation and the code was made available to the laboratory only after the last sample was analyzed. Both CSF-CRH and CSF-SRIF were analyzed using sensitive and specific immunoassay procedures described earlier (Nemeroff et al., 1984; Bissette et al., 1986).

All 24 patients were followed up regularly after discharge as outpatients, within the same psychia-

tric hospital. Despite continuing antidepressant medication and regular visits nine patients suffered a depressive relapse within the first six months, severe enough to require at least a few days' rehospitalization. The remaining 15 women remained depression-free during the 6-month follow-up period and they received a maintenance dose of their respective antidepressant medication continuously.

Pre- and post-treatment CSF neuropeptide data were compared with paired *t*-tests; the difference between relapsed and nonrelapsed groups was evaluated by analysis of covariance. Correlations with neuropeptide concentrations and background variables were computed by Pearson's coefficients, and their effect on the peptide concentration was calculated by multiple regression analysis. Baseline CRH and SRIF data were log-transformed for these calculations because they appeared to be log-normally distributed as observed earlier (Banki et al., 1987).

Results

Mean \pm SD of each variable, together with the range, is given in Table 2. where both ΔCRH and ΔSRIF are calculated as the difference between the post- and pre-treatment value (minus difference indicating decrease). Difference values appeared to be approximately normally distributed for both neuropeptides; the mean difference of CSF-CRH in the total group was -5.6 ± 29.0 ($t = 0.94$, d.f. = 23, N.S.), and the mean difference of CSF-SRIF was 3.3 ± 13.5 pg/ml ($t = 1.19$, d.f. = 23, N.S.).

Figures 1 and 2 present individual CSF-SRIF and CSF-CRH data pairs of patients, separately in the relapsed and nonrelapsed subgroups. While there was no significant difference in either subgroup and between the subgroups for the log-SRIF concentration before and after treatment

TABLE 2
MEANS AND STANDARD DEVIATIONS OF BASELINE NEUROPEPTIDES AND CHANGES DURING ANTIDEPRESSANT TREATMENT

	pg/ml
CRH	66.1 ± 38.8 (19–178)
ΔCRH	-5.6 ± 29.0 (–73–50) ^a
SRIF	27.8 ± 13.1 (8–51)
ΔSRIF	3.3 ± 13.5 (–22–37) ^a

^aCalculated as post-treatment minus pre-treatment difference.

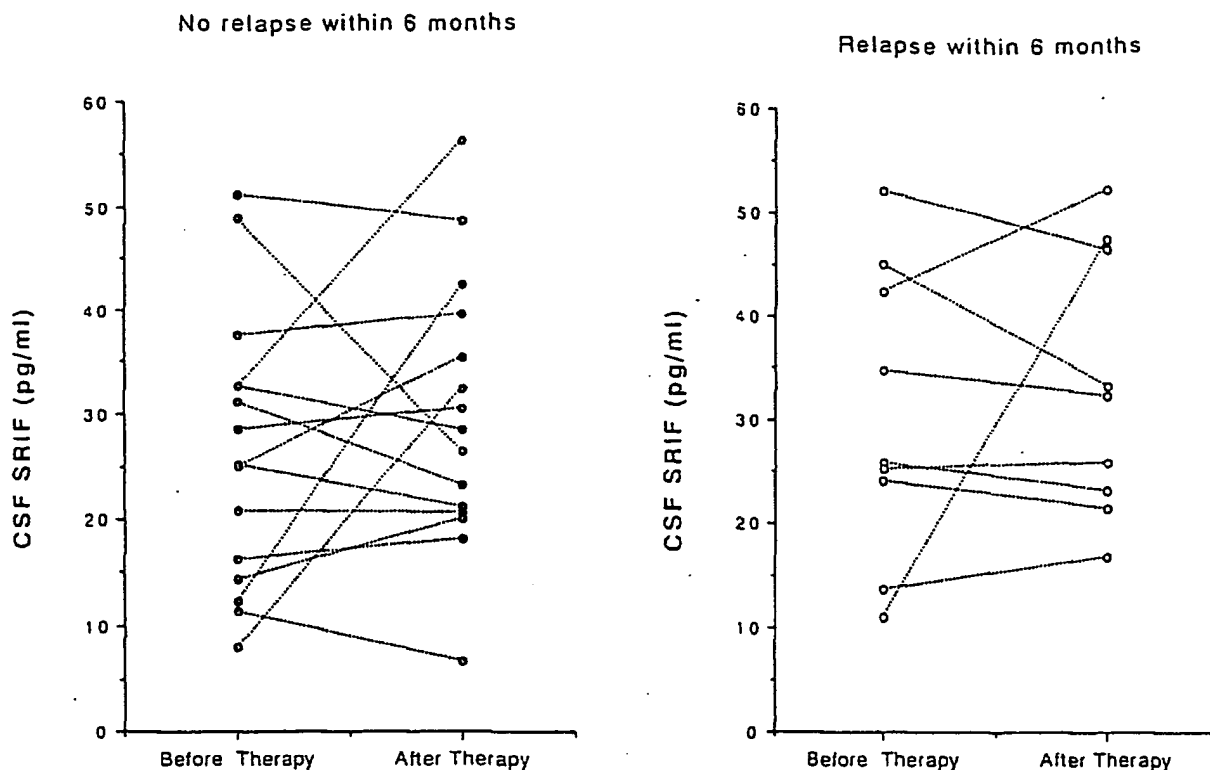


Fig. 1. CSF somatostatin changes during antidepressant treatment in patients with and without early relapse. The response was not different between the subgroups (ANCOVA $F(1,21) = 0.47$, $P > 0.50$, N.S.).

(ANCOVA, $F(1,22) = 0.47$, N.S.), there was a significant difference between the log-CRH responses of the subgroups (ANCOVA $F(1,21) = 8.83$, $P < 0.008$). As can be seen in Fig. 2, CSF-CRH values in the non-relapsed subgroup decreased significantly ($t = 2.58$, d.f. = 14, $P < 0.025$) while they tended to increase in the relapsed patients ($t = 1.48$, d.f. = 8, N.S.).

We registered several clinical variables in our patients and computed their correlations with both baseline and treatment-induced neuropeptide change values. The correlation coefficients, using log-transformation for CRH and SRIF, are presented in Table 3. It is noteworthy to emphasize the significant negative correlations of both baseline neuropeptide concentrations with body height, and the positive correlation of CSF-CRH with age. No other significant coefficient was found. Based on these variables we computed multiple regression analysis to see whether CSF neuropeptide concentrations were significantly dependent on these variables: none of these analyses resulted in significant R^2 -values (CRH: 0.601; Δ CRH: 0.512; SRIF: 0.465; Δ SRIF: 0.383).

The highest multiple determination coefficient was seen for baseline CSF-CRH and its only significant beta was for body height. However, the significance of these few findings among the large number of coefficients calculated for a small patient sample must remain uncertain.

There was no significant difference for any neuropeptide measurement between patient subgroups receiving different antidepressants; there

TABLE 3
CORRELATIONS BETWEEN NEUROPEPTIDE AND CLINICAL VARIABLES

	CRH	Δ CRH	SRIF	Δ SRIF
Age	0.41*	-0.24	0.31	0.05
Weight	-0.08	0.27	0.01	0.27
Height	-0.47*	0.10	-0.50*	0.21
No. episodes	-0.11	0.20	-0.02	-0.10
Time/onset	-0.06	0.37	0.05	-0.16
Epis. duration	-0.06	0.00	0.38	-0.36
G.A.F.	-0.23	0.11	0.08	-0.05
HAMD	0.23	-0.11	-0.06	0.10
Melancholia	0.23	0.09	-0.16	0.31

* $P < 0.05$.

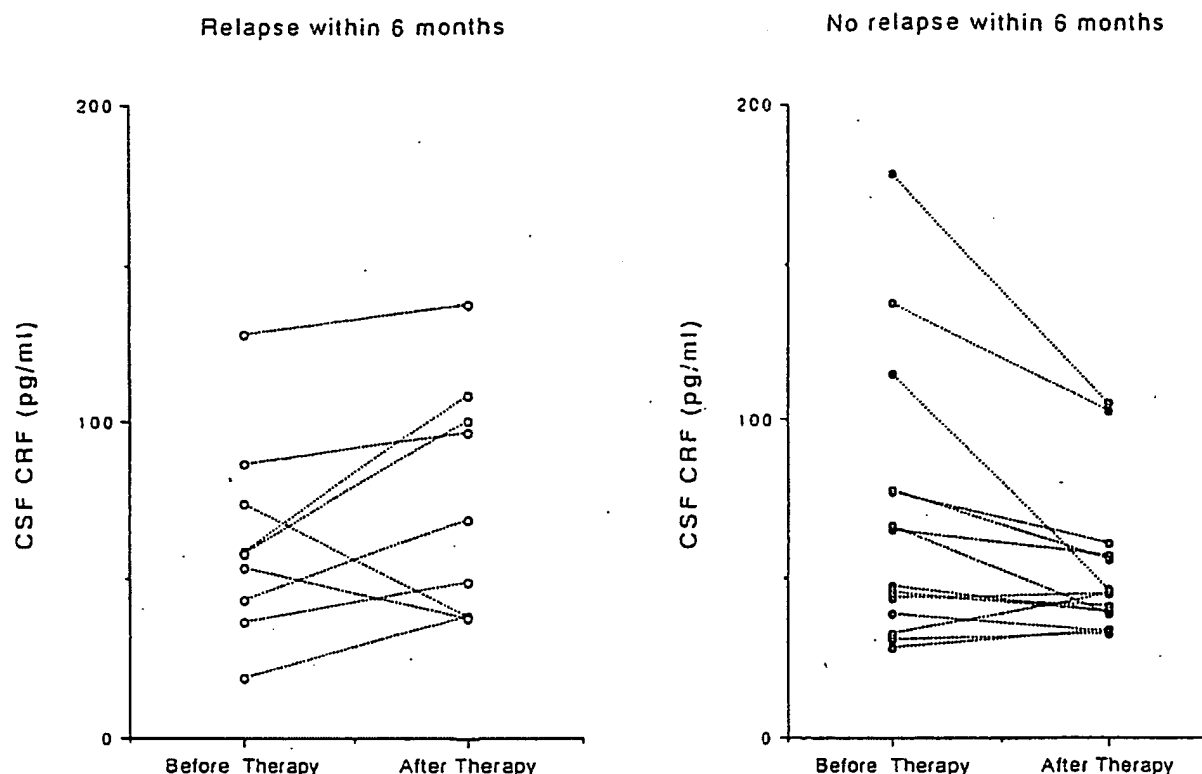


Fig. 2. CSF corticotropin-releasing hormone changes during antidepressant treatment in patients with and without early relapse. The response was significantly different between the subgroups (ANCOVA, $F(1,21) = 8.83$, $P < 0.008$).

was no difference for the 2 subjects who received ECT (neither of them relapsed within 6 months) as compared to those who received only drugs; and there was no difference between the melancholic and non-melancholic patients in their peptide responses. No correlation between the final HAMD score or the HAMD difference score and either CRH or SRIF responses were found. Finally, no correlation between duration of treatment (between 35 and 54 days) and CSF neuropeptide levels could be demonstrated.

Discussion

Mean baseline CSF-CRH concentration in this population of major, mostly melancholic and/or psychotic depressed women was above 66 pg/ml which is similar to our earlier observations (Banki et al., 1987). None of these patients has been included in any other previous reports, thus indicating reproducibility of the findings (and of the interassay characteristics of our CRH measurement procedure as well). In the discordant study by

Roy et al. (1987) the controls had remarkably similar mean (and also SD) values of CSF-CRH as our controls; the higher peptide concentrations in our depressed patients may be related to their more advanced age (which may be correlated to CSF-CRH), more severe depression, shorter hospitalization before LP, or less exposure to antidepressant drugs in the preceding weeks or months, but these explanations remain speculative in the absence of between-country comparative studies of depressed in-patients. Baseline SRIF concentrations were found to be somewhat lower than the mean values reported from other laboratories (Post et al., 1988) but still in the same range and with very similar standard deviation.

Both neuropeptide concentrations were found to be significantly correlated with body height. This phenomenon, not seen earlier (Banki et al., 1987) may be due to similar factors which underlie the same relationship seen with the monoamine metabolites in the CSF; in fact, there is a significant cranio-caudal gradient for CSF-CRH (Arato et al., 1989) and probably for SRIF as well (Rubinow et al., 1984). The positive correlation

between CSF-CRH and age may be limited to depressed women, or to an older age group, because it was not seen earlier in controls and it was not seen in other patient populations (Nemeroff et al., 1984; Roy et al., 1987). Our specific population excluded the investigation of sex differences.

The most important finding was the absence of significant reduction in CSF-CRH concentration in the total group despite the clinically significant, marked symptomatic improvement. This is different from the short-time effect of ECT on CSF-CRH (Nemeroff et al., 1991) and indicates a dissociation between the neuropeptide release into the CSF and the presence of psychological symptoms of depression. A possible confounding factor is the largely unknown direct effect of antidepressant drugs on central CRH release; but it is noteworthy that there was no difference between the effects of a selective noradrenaline reuptake blocker (maprotiline) and the nonselective drugs (dibenzepine and amitriptyline) on the CSF-CRH. This may indicate that CSF-CRH changes are more associated with the drug-induced longterm adaptive processes than with the immediate reuptake blocking. The other major finding in this study, namely the lack of reduction of CSF-CRH levels being predictive on an early depressive relapse, corroborate this assumption. It supports earlier findings with the dexamethasone suppression test (Greden et al., 1980; Carroll, 1982) where likewise normalization occurred only in a subgroup of patients but this was found to be a good prognostic sign. With the CRH/ACTH test, normalization with recovery was the rule (Gold et al., 1984) but it did not occur in each case; whether the non-normalization predicts relapse needs confirmation in larger patient samples. There is some recent evidence from preclinical work that chronic glucocorticoid treatment may induce catecholaminergic and serotonergic changes both pre- and post-synaptically that resemble changes assumed to develop in human depression (Szemerédi et al., 1988; Bagdy et al., 1989; Calogero et al., 1990). Along this line it can be hypothesized that an underlying HPA overactivity (of which CSF-CRH concentration may be one measure) represents a primary abnormality while monoaminergic changes that are directly influenced by antidepressant drugs are 'secondary' phenomena more closely associated with the psychological symptoms. In this respect the more uniform response of CRF-CRH to ECT corresponds to its different mechanism

of action (Fink and Ottoson, 1980) in particular on the peptidergic processes.

We did not observe a significant elevation, ie. normalization, of CSF-SRIF during antidepressant drug treatment. This was absent both in the relapsing and the nonrelapsing patients. Remarkably, no significant SRIF elevation was found after ECT either (Nemeroff et al., 1991). Earlier studies found 'normal' mean SRIF levels in affective patients in the euthymic state but also described no effect of common antidepressants on the CSF-SRIF concentration (Rubinow et al., 1984). Selective serotonergic antidepressants may elevate SRIF while carbamazepine may decrease it, but we used neither drugs in this study. Whether normalization occurs after a longer euthymic period remains to be investigated.

In summary, we found no consistent changes of either CSF-CRH or CSF-SRIF in response to a clinically successful antidepressant drug treatment during 5–8 weeks. However, patients who relapsed within 6 months could be separated by their non-decreasing CSF-CRH concentrations from those who remained symptom-free and showed significant reduction of their initially elevated CRH values during treatment. Although CSF neuropeptide measurements are unlikely to become common tools in psychiatric practice they represent an important research tool to understand the underlying pathophysiology of major depression.

References

- Ågren, H. and Lundqvist, G. (1984) Low levels of somatostatin in human CSF mark depressive episodes. *Psychoneuroendocrin.* 9, 233–248.
- Arato, M., Banki, C.M., Bissette, G. and Nemeroff, C.B. (1989) Elevated CSF CRF in suicide victims. *Biol. Psychiat.* 25, 355–359.
- Bagdy, G., Calogero, A.E., Chrousos, G.P. and Szemeredy, K. (1989) Delayed effects of chronic cortisol treatment on brain and plasma concentrations of corticotropin (ACTH) and beta-endorphin. *Brain Res.* 489, 216–222.
- Banki, C.M., Bissette, G., Arato, M., O'Connor, L. and Nemeroff, C.B. (1987) CSF corticotropin-releasing factor-like immunoreactivity in depression and schizophrenia. *Am. J. Psychiat.* 144, 873–877.
- Bissette, G., Spielman, F., Stanley, M., Banki, C.M., Fink, M., Stanley, B., Golden, R.I. and Nemeroff, C.B. (1985) Further studies of corticotropin-releasing factor-like immunoreactivity in patients with affective disorders. *Soc. Neurosci. Abstr.* 11, 133.
- Bissette, B., Widerlöv, E., Walleus, H. and Nemeroff, C.B. (1986) Alterations in CSF concentrations of somatostatin-like immunoreactivity in neuropsychiatric disorders. *Arch. Gen. Psychiat.* 43, 1148–1154.

- Calogero, A.E., Bagdy, G., Szemeredy, K., Tartaglia, M.E., Gold, P.W. and Chrousos, G.P. (1990) Mechanism of serotonin receptor agonist-induced activation of the hypothalamic-pituitary-adrenal axis in the rat. *Endocrinology* 126, 1888-1894.
- Carroll, B.J. (1982) The dexamethasone suppression test for melancholia. *Br. J. Psychiat.* 140, 292-304.
- Carroll, B.J. (1985) Dexamethasone suppression test: a review of contemporary confusion. *J. Clin. Psychiat.* 46, 13-24.
- Doran, A.R., Rubinow, D.R., Roy, A. and Pickar, D. (1986) CSF somatostatin and abnormal response to dexamethasone administration in schizophrenia and depressed patients. *Arch. Gen. Psychiat.* 43, 365-369.
- Fink, M. and Ottoson, J.O. (1980) A theory of convulsive therapy in endogenous depression: significance of hypothalamic functions. *Psychiat. Res.* 2, 49-61.
- Gold, P.W., Chrousos, G., Kellner, C., Post, R., Roy, A., Augerinos, P., Oldfield, E. and Loriaux, D.L. (1984) Psychiatric implications of basic and clinical studies with corticotropin-releasing factor. *Am. J. Psychiat.* 141, 619-627.
- Gold, P.W. and Chrousos, G.P. (1985) Clinical studies with corticotropin-releasing factor: implications for the diagnosis and pathophysiology of depression, Cushing's disease, and adrenal insufficiency. *Psychoneuroendocrinology* 10, 401-419.
- Greden, J.P., Albala, A., Haskett, R.F. and Carroll, B.J. (1980) Normalization of dexamethasone suppression test: a probable index of recovery among endogenous depressives. *Biol. Psychiat.* 15, 449-458.
- Halbreich, U., Asnis, G.M., Schindlacker, R., Zumoff, B. and Nathan, R.S. (1985) Cortisol secretion in endogenous depression. *Arch. Gen. Psychiat.* 42, 904-908.
- Holsboer, F. (1988) Implications of altered limbic-hypothalamic-pituitary-adreno-cortical (LHPA) function for neurobiology of depression. *Acta Psychiat. Scand.* 77 (Suppl. 341), 72-111.
- Koob, G.F. and Bloom, F.E. (1985) Corticotropin-releasing factor and behavior. *Fed. Proc.* 44, 259-263.
- Loosen, P.T. and Banki, C.M. (1988) The use of nonopioid neuropeptides as diagnostic tools in psychiatric and neurological disorders. In: Nemeroff, C.B. (Ed.), *Neuropeptides in Psychiatric and Neurological Disorders*, Johns Hopkins Univ. Press, Baltimore, MD, pp. 18-48.
- Nemeroff, C.B., Widerlöv, E., Bissette, G., Walleus, H., Carlsson, I., Eklund, K., Kiltz, C.D. Loosen, P.T. and Vale, W. (1984) Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* 226, 1342-1344.
- Nemeroff, C.B., Walsh, T.J. and Bissette, G. (1987) Somatostatin and behavior: preclinical and clinical studies. In: Reichlin, S. (Ed.), *Somatostatin*, Plenum, New York, pp. 157-167.
- Nemeroff, C.B., Owens, M.J., Bissette, G., Andorn, A.C. and Stanley, M. (1988) Reduced corticotropin-releasing factor binding sites in the frontal cortex of suicide victims. *Arch. Gen. Psychiat.* 45, 577-579.
- Nemeroff, C.B. (1988) The role of corticotropin-releasing factor in the pathogenesis of major depression. *Pharmacopsychiatry* 21, 76-82.
- Nemeroff, C.B., Bissette, G., Akil, H. and Fink, M. (1991) Neuropeptide concentrations in the CSF of depressed patients treated with electroconvulsive therapy. *Br. J. Psychiat.* 158, 59-63.
- Post, R.M., Rubinow, D.R. and Gold, P.W. (1988) Neuropeptides in manic-depressive illness. In: Nemeroff, C.B. (Ed.), *Neuropeptides in Psychiatric and Neurological Disorders*, Johns Hopkins Univ. Press, Baltimore, MD, pp. 76-115.
- Roy, A., Pickar, D., Paul, S., Doran, A., Chrousos, G.P. and Gold, P.W. (1987) CSF corticotropin-releasing hormone in depressed patients and normal control subjects. *Am. J. Psychiat.* 144, 641-645.
- Rubinow, D.R., Gold, P.W., Post, R.M., Ballenger, J.C. and Cowdry, R.W. (1984) Somatostatin in patients with affective illness and in normal volunteers. In: Post, R.M. and Ballenger, J.C. (Eds.), *Neurobiology of Mood Disorders*, Williams and Wilkins, Baltimore, MD, pp. 369-387.
- Szemeredy, K., Bagdy, G., Stull, R., Calogero, A.E., Kopin, I.J. and Goldstein, D.S. (1988) Sympathoadrenomedullary inhibition in chronic glucocorticoid treatment in conscious rats. *Endocrinology* 123, 2585-2590.



Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated

Astrid W. Zobel, Thomas Nickel, Heike E. Künzel, Nibal Ackl, Annette Sonntag,
Marcus Ising, Florian Holsboer*

Max Planck Institute of Psychiatry, Kraepelinstr. 2-10, D-80804 Munich, Germany

Received 1 March 2000; received in revised form 20 April 2000; accepted 25 April 2000

Abstract

Clinical and preclinical data suggest that unrestrained secretion of corticotropin-releasing hormone (CRH) in the CNS produces several signs and symptoms of depression and anxiety disorders through continuous activation of CRH₁ receptors. This led to the development of drugs that selectively antagonize CRH₁ receptors suppressing anxiety-like behavior in rats and also in monkey models of anxiety. These findings led to a clinical development program exploring the antidepressive potential of R121919, a water-soluble pyrrolopyrimidine that binds with high affinity to human CRH₁ receptors and is well absorbed in humans. This compound was administered to 24 patients with a major depressive episode primarily in order to investigate whether its endocrine mode of action compromises the stress-hormone system or whether other safety and tolerability issues exist. The patients were enrolled in two dose-escalation panels: one group ($n = 10$) where the dose range increased from 5–40 mg and another group ($n = 10$) where the dose escalated from 40 to 80 mg within 30 days each. Four patients dropped out because of withdrawal of consent to participate (three cases) or worsening of depressive symptomatology in one case. We found that R121919 was safe and well tolerated by the patients during the observation period. Moreover, the data suggested that CRH₁-receptor blockade does not impair the corticotropin and cortisol secretory activity either at baseline or following an exogenous CRH challenge. We also observed significant reductions in depression and anxiety scores using both, patient and clinician ratings. These findings, along with the observed worsening of affective symptomatology after drug discontinuation, suggests that the pharmacological principle of CRH₁-receptor antagonism has considerable therapeutic potential in the treatment and the prevention of diseases where exaggerated central CRH activity is present at baseline or following stress exposure. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Corticotropin-releasing hormone (CRH) has been identified as a neuropeptide that plays a central role in the coordination of neuroendocrine, autonomic and behavioral responses to stress (Vale et al., 1981). Once released from the hypothalamic paraventricular nucleus it enters the portal vessels via the median eminence to stimulate synthesis of proopiomelanocortin,

the precursor of corticotropin (ACTH). In response to stress exposure this neuropeptide hormone is secreted into the circulation and stimulates the synthesis and release of adrenal corticosteroids which in turn suppress the synthesis of both hypothalamic CRH and corticotrophic ACTH in order to reinstate homeostasis of the hypothalamic–pituitary adrenocortical (HPA) system (Plotsky, 1991). The setpoint which defines an individual's HPA homeostasis is determined by genetic as well as environmental factors, particularly traumatic events in early life (Coplan et al., 1996). If the setpoint is at a high level, negative feedback control upon hypothalamic CRH synthesis and release is decreased.

* Corresponding author. Tel.: 089-30622-220; fax: 089-30622-483.
E-mail address: Holsboer@mpipsykl.mpg.de (F. Holsboer).

This results in a continuous hyperactivity of CRH neural circuits directly or indirectly interconnecting the hypothalamic paraventricular nuclei (PVN) with extra-hypothalamic sites supposed to play an important role in the mediation of behavioral response to stress (for a review see Keck and Holsboer, 2000). For example, the locus coeruleus, a brainstem nucleus from which noradrenergic neurons project to the forebrain, contains CRH immunoreactive fibers and is activated by CRH (Valentino et al., 1993). Also, the central amygdala thought to mediate fear and anxiety (Davis, 1992), is innervated by CRH nerve terminals, and in this brain region in rodents CRH gene expression is thought to be activated by corticosteroids (Schulkin et al., 1998). Thus, impaired negative feedback by corticosteroids enhances release of CRH from the PVN which further increases corticosteroids via elevated ACTH. At the level of the amygdala this elevation of corticosteroids may even enhance CRH gene expression, possibly producing anxiety-like behavior. Other behavioral phenomena associated with unrestrained CRH include disturbed sleep, loss of sexual drive, psychomotor and autonomic changes and decreased appetite. These behavioral changes can also be induced by central administration of CRH in rats and monkeys (Kalin, 1985) or by inserting a CRH gene in the mouse genome resulting in CRH overproducing transgenic mice (Stenzel-Poore et al., 1994). All of the CRH-elicited effects resemble the clinical signs and symptoms characteristic for patients with severe depression (Owens and Nemeroff, 1991; Holsboer et al., 1992). These patients also show hyperactivity of the HPA system and experimental evidence has shown that a hyperactive CRH system is a major cause for this neuroendocrine disturbance. Clinical studies have also demonstrated elevated baseline ACTH and cortisol secretion, and their inadequate suppression by dexamethasone, a synthetic glucocorticoid (for a review see Holsboer, 1995). Further, CRH concentrations are elevated in the cerebrospinal fluid of depressed patients (Nemeroff et al., 1984), which, if extrapolated to the situation in the brain, is consistent with reduced CRH binding in forebrains of depressed suicide victims (Nemeroff et al., 1988) and elevated numbers of CRH-producing neurons in the PVN of patients with depression (Raadsheer et al., 1994). Finally, the ACTH response to human and ovine CRH was found to be blunted among depressed patients indicating desensitized CRH receptors secondary to central hypersecretion (Gold et al., 1984; Holsboer et al., 1984, 1986). These clinical data, and the behavioral data derived from manipulations of the CRH system in animals, are consistent with exaggerated CRH secretion as a causal mechanism accounting not only for the neuroendocrine but also for psychopathological symptoms of depression and anxiety dis-

orders (Holsboer, 1999). Since the CRH signal is mediated through different CRH receptors localized in different regions in the rat, mouse and human brain (Chalmers et al., 1996), it was important to know which of these two identified CRH receptors would be an appropriate target for a drug reducing the potentially depressogenic and anxiogenic effects of CRH. Studies using antisense oligodeoxynucleotides directed against the mRNA of CRH₁ and CRH₂ receptors, as well as mouse mutants where CRH₁ receptors were lacking, supported the hypothesis that stress-induced anxiety-like behavior is mediated through the CRH₁ receptors (Liebsch et al., 1995, 1999; Heinrichs et al., 1997; Skutella et al., 1994, 1998; Smith et al., 1998; Timpl et al., 1998; Steckler and Holsboer, 1999). This concept led pharmaceutical companies to screen compound libraries for molecules that might act as CRH₁ receptor antagonists and have properties suitable for clinical drug use (Chen et al., 1993; Schulz et al., 1996; Grigoriadis and de Souza, 1998; Shaham et al., 1998). One of these candidates is R121919 (formerly NBI 30775), a pyrrolopyrimidine, which is well absorbed when given orally, penetrates the blood-brain barrier and binds to cloned human CRH₁ receptors with high affinity ($K_i < 3$ nM) — binding to other neurotransmitter and neuropeptide receptors or transporters was absent or greater than 1000-fold different. Given subcutaneously to rats this compound antagonizes several behavioral effects induced by CRH pretreatment or by CRH overexpression in transgenic mice (Steckler et al., unpublished observation). In rats selectively bred for high anxiety-like behavior (Liebsch et al., 1998) the CRH₁-receptor antagonist R121919 blocked CRH binding to CRH₁ receptors and exerted anxiolytic effects in a dose-dependent manner in these rats (Keck et al., unpublished observation). Comparable anxiolytic effects of R121919 were absent in rats that were selectively bred for low anxiety. These preclinical observations prompted us to conduct an open-label trial in patients with major depression to get some initial information as to how this class of compounds might affect neuroendocrine and safety parameters and whether it can be well tolerated in this clinical condition. Although designed as a safety and tolerability study, not allowing definitive conclusions about efficacy to be made, we were also interested to observe whether specific changes in psychopathology might emerge during treatment with R121919.

2. Methods

Over the course of 13 months (December 1998 to December 1999) 24 patients were subsequently selected from referrals to the Clinical Department of the Max Planck Institute of Psychiatry for treatment of a major

depressive episode and enrolled into the study, provided they fulfilled the inclusion criteria. The study was conducted according to the regulations of the state of Bavaria (Federal Republic of Germany) and the Declaration of Helsinki which includes approval of the local ethical committee. Written informed consent was collected from all patients after the purpose and the experimental details and risks were explained and before protocol-specified procedures were initiated (see Fig. 1). Patients were informed that they could withdraw their consent at any time during the study without any justification and that the privacy of their medical records not related to the study protocol would be protected. Patients were required to have scores equal to or above 18 on the 21-item Hamilton Depression Rating Scale (HAMD) at screening and at day 0. Other exclusion criteria were a history or suspicion of substance abuse, specifically of alcohol, benzodiazepines, barbiturate, amphetamine and narcotics.

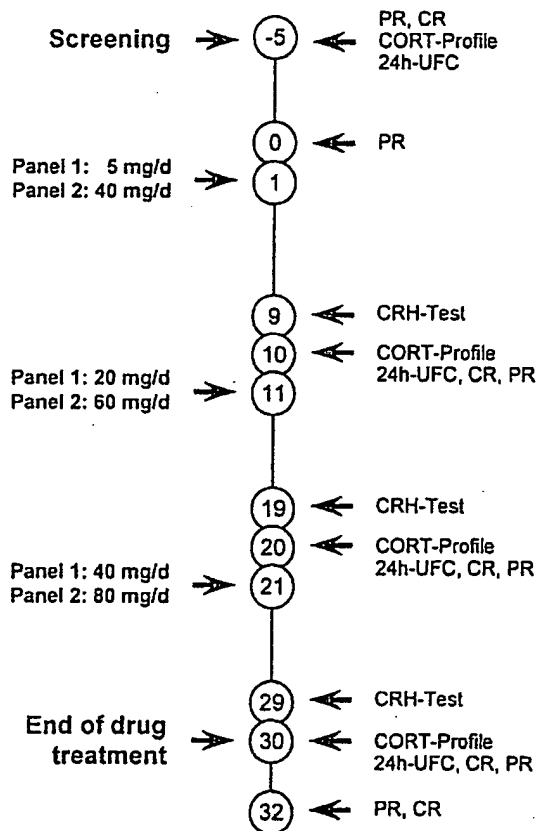


Fig. 1. Study design of both panels which only differ in the dose escalations. Abbreviations: PR=psychopathology rating (HAMD, HAMA, CGI, BDI, STAI); CR=clinical routine; CORT profile=venous blood samples taken at 0, 3, 8, 12 and 24 h for plasma ACTH and cortisol analysis; 24 h UFC=urinary free cortisol analysis in a 24-h urine collection.

Patients were not admitted if they had a serious or unstable medical illness including cardiovascular, rheumatoid, hepatic, renal, respiratory, metabolic, neurological or hematological disease. Specifically excluded were patients with endocrine disorders such as clinical or laboratory evidence for thyroid disease, Cushing's syndrome or Addison's disease. Women of child-bearing age were excluded even if contraceptive means were applied. Patients who posed a current suicidal risk were excluded, as were patients who were judged as treatment resistant, arbitrarily defined as non-responding to standard antidepressants at therapeutic dosages for at least 6 months. During the screening period all psychoactive medication was stopped for a minimum of 5 days and only chloralhydrate up to 2×500 mg/day was allowed as a sleeping aid. Patients having taken drugs known to be hepatic enzyme inducers, i.e. carbamazepine, barbiturates, phenytoin, cimetidine etc., were not enrolled. Pretreatment with monoamine oxidase inhibitors, fluoxetine or slow-release neuroleptics had to be discontinued for at least 1 month and treatment with corticosteroids or electroconvulsion within 3 months prevented study inclusion. Following screening, when all inclusion and exclusion criteria were defined, active treatment was initiated applying the two different dose-escalation regimens. For safety reasons the first 10 patients were recruited into panel 1 administering the lower dosages before panel 2 was started. Details of the dose escalation and the time points when psychopathometric assessments, clinical examinations and neuroendocrine tests were applied are illustrated in Fig. 1.

The laboratory analysis of clinical chemistry, hematology and hormone data was performed according to standard procedures. The circadian hormone secretion was estimated by ACTH and cortisol analysis in blood specimens at the time points given in Fig. 1. Total 24-h cortisol secretion was assessed by measuring free cortisol in a 24-h urine sample (UFC). Dose regimens for both panels are depicted in Fig. 1. The CRH test employed a protocol that was similar to that previously described (Holsboer et al. 1986): at 1400 h an indwelling catheter was inserted into a forearm vein and kept patent by a slow drip (50 ml/h) of physiological saline solution. At 14.00 h, 14.30 h, 14.45 h and 15.00 h a 3-ml venous blood sample was collected to assess baseline HPA activity. At 15.00 h a bolus of 100 µg of human CRH (Ferring, Germany) was injected through the catheter and samples were collected at the time points given in Fig. 2.

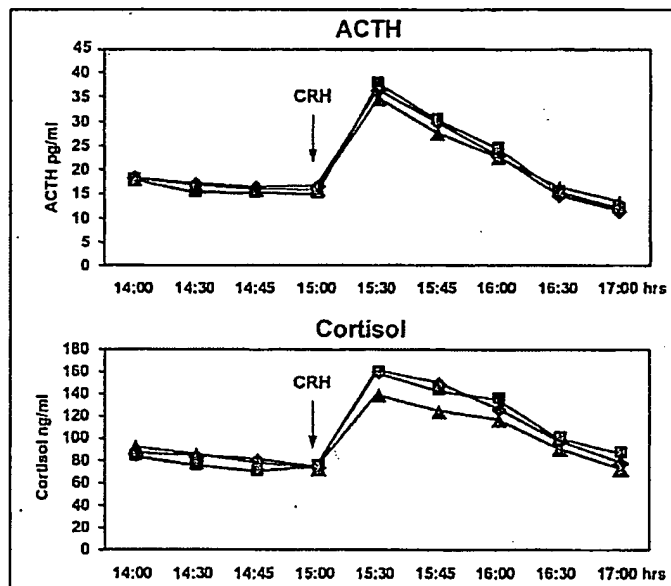
2.1. Data analysis

Differences within and between both dosing panels were assessed by methods based on analysis of variance. Within each panel the degree of

changes in symptom severity between screening and the consecutive days when tested under treatment [i.e. HAMD, Beck Depression Inventory (BDI), Hamilton Scale for Anxiety (HAMA), State Trait Anxiety Inventory (STAI), and Clinical Global Impression (CGI)] was estimated by deviation con-

trasts. These measures correspond to two-tailed paired *t*-test analyses. Because of the small sample size non-parametric Kendall's Tau coefficients were applied for correlational analyses. Construct-homogeneous correlations (i.e. depression related: HAMD, BDI; anxiety related: HAMA, STAI) were compared

Panel 1



Panel 2

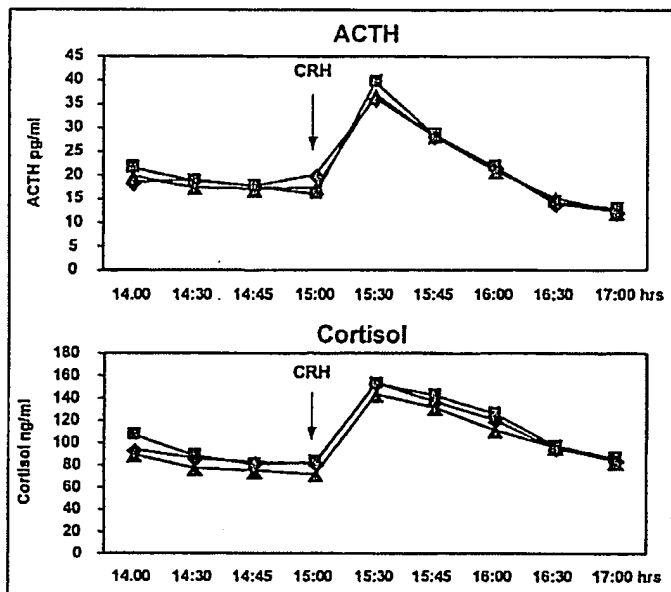


Fig. 2. Time-course curves of plasma ACTH and cortisol concentrations prior to and following an intravenous injection of 100 µg human CRH at 15:00 h at day 9 (diamond), day 19 (square) and day 29 (triangle) during panel 1 and panel 2. If CRH-stimulated plasma ACTH and cortisol output is expressed as the area under the time-course curve (AUC), no suppressive effect of R121919 on AUC values is noted at any time point at any dose given.

with construct-divergent correlations (HAMD/STAI and HAMA/BDI).

Effects of drug treatment upon neuroendocrine parameters employed deviation contrasts to assess the degree of change in hormonal secretions among patients treated in both panels. Analysis of effects of the drug upon circadian rhythmicity used a quadratic trend coefficient, reflecting the U-shaped circadian rhythm. The area under the time-course curve (AUC) for ACTH and cortisol, following CRH injections, was calculated according to a trapezoidal rule, and from this value the mean of cortisol levels calculated from the plasma ACTH and cortisol concentrations prior to CRH injection was subtracted. Again for correlational analyses, non-parametric Kendall's Tau coefficients were applied. But, under the perspective of outcome prediction, non-parametric Spearman's Rho coefficients were used because they are likely to be more appropriate in the case of a dichotomous outcome, which is treatment response vs treatment nonresponse. As neuroendocrine predictor variables urinary free cortisol (UFC), mean plasma cortisol, and ACTH concentration at screening day, and AUCs of ACTH and

cortisol following CRH infusion at day 9 were employed (see Fig. 1).

The data analysis presented here was based upon 20 patients that had completed the study. We also inspected what effect the inclusion of drop-out patients would have had if they were not prematurely discharged from the study and if their clinical condition at the time of drop-out would not have changed until termination of the study. This data analysis used the intent-to-treat approach and is presented in order to rule out the possibility that the differences between both dose-escalation panels were merely due to the drop-out cases in panel 2.

3. Results

3.1. Patients description

As documented in Table 1, there were no major differences between the patients in panel 1 and panel 2 with regard to gender, age, diagnostic attributions, length of index episode, family history and pretreatment. This allows comparison of drug effects regarding

Table 1

Demographic and clinical data of 20 completers (10 in panel 1 and 10 in panel 2) and of four patients who dropped out from panel 2 before the end of the trial. Patient (BDI and STAI) and clinician (HAMD, HAMA and CGI) rating scores before and after treatment with R121919 are also given (mean \pm SD). Symptoms are listed which worsened among six patients in both panels after discontinuation of the drug (see also Table 4)

	Panel 1 (10 patients)	Panel 2 (10 patients)	Drop out (4 patients)
Sex	6 males 4 females	5 males 5 females	2 males 2 females
Age	43.8 \pm 11.4	50.7 \pm 13.0	36.8 \pm 10.3
DSM IV 296.22	–	1 patient	–
DSM IV 296.23	3 patients	2 patients	1 patient
DSM IV 296.32	4 patients	3 patients	3 patients
DSM IV 296.33	3 patients	4 patients	–
Index episode (weeks)	18.2 \pm 13.7	18.0 \pm 6.6	23.5 \pm 19.6
Positive family history	8 patients	8 patients	2 patients
Pretreated with antidepressants	7 patients	7 patients	2 patients
HAMD screening	26.2 \pm 3.6	27.6 \pm 7.4	23.3 \pm 6.0
HAMD last day of medication	15.4 \pm 9.0	11.0 \pm 8.0	21.0 \pm 8.1 ^a
BDI screening	27.1 \pm 8.8	27.2 \pm 11.2	21.0 \pm 10.0
BDI last day of medication	21.1 \pm 10.1	12.9 \pm 6.2	22.8 \pm 6.7 ^a
HAMA screening	23.0 \pm 4.1	26.6 \pm 9.8	21.0 \pm 5.7
HAMA last day of medication	13.9 \pm 10.5	10.1 \pm 9.5	18.8 \pm 5.6 ^a
STAI screening	61.6 \pm 10.2	65.1 \pm 8.9	55.5 \pm 12.0
STAI last day of medication	57.8 \pm 14.8	51.8 \pm 8.6	63.0 \pm 5.9 ^a
CGI screening	3.7 \pm 0.5	4.1 \pm 1.0	3.75 \pm 0.5
CGI last day of medication	2.8 \pm 0.9	2.2 \pm 1.0	3.25 \pm 1.0 ^a
Remitter HAMD \leq 8	3 patients	6 patients	–
Responder HAMD \geq 50%	5 patients	8 patients	–
Nonresponder	5 patients	2 patients	–
After cessation of medication worsening of symptoms	6 patients (agitation, mood, sleep, somatic symptoms)	6 patients (agitation, mood, sleep, somatic symptoms)	–

^a Psychopathology ratings of drop outs were derived from the last visit carry over approach.

safety and tolerability measures as well as psychopathometric scores.

3.2. Laboratory tests

Clinical chemistry, hematology ECG and EEG recordings yielded no adverse effects that could be specifically attributed to R121919. None of the 10 patients enrolled in panel 1 had elevated liver enzyme values during treatment with R121919. However, when shifted to mirtazapine, in five of these 10 patients liver enzyme values increased. In panel 2 we observed slightly increased liver transaminases during treatment with R121919 in three cases. The maximum values

reached in panel 2 were: AST: 45 U/l (normal range: 0–18 U/l); ALT: 80 U/l (normal range: 0–22 U/l); and GGT: 47 U/l (normal range: 6–28 U/l). After discontinuation of R121919 and the subsequent treatment of the patients with mirtazapine the liver enzyme values normalized in one patient and further increased in two other cases. In none of the cases did R121919 induce elevations of bilirubine. Heart rate tended to decrease during treatment in both panels and a small rebound was noted after drug cessation, however, no significant differences in heart rate between both panels emerged.

3.3. Endocrine effects

Comparison of UFC values yielded no significant difference between both panels at any time point. When UFC values during treatment were compared with UFC values at screening, a trend towards a steeper decline of UFC values was found in panel 2, where significant differences emerged at day 20, while significant differences in panel 1 were not observed until day 30.

In both dose-escalation panels no significant effects of the drug upon plasma cortisol, ACTH and renin concentrations were observed at any time. Moreover, no effects of the drug upon circadian rhythms of ACTH and cortisol emerged. When the plasma cortisol concentrations of all five sampling times were averaged, a trend for reduced mean cortisol values was observed across both panels. However, this cortisol lowering effect was not different between the two panels. Such trends were also not observed on plasma ACTH measurements. In addition, the CRH-elicited plasma ACTH and cortisol secretions were not different between both panels at any time point (see Fig. 2).

3.4. Psychopathometric findings

As illustrated in Fig. 3 HAMD rating scores dropped significantly in both panels. When these were analysed separately the differences between the mean HAMD values at each time point compared with the values at screening (baseline) were more marked in panel 2 (see also Tables 1 and 2). When a reduction of the HAMD score of $\leq 50\%$ of the score at screening was defined as criterion for response, five patients in panel 1 were identified as responders, three of whom were also remitters (i.e. they had a HAMD score of ≤ 8 points at day 30). In panel 2, eight out of 10 patients met the criterion of response, and six of these were also remitters (see Table 1). Notably, four patients of the total group of 14 enrolled in panel 2 dropped out before completing the 30-day study because of withdrawal of consent to participate in three cases at study days 13, 19 and 20, and a worsening of depressive symptomatology, including current

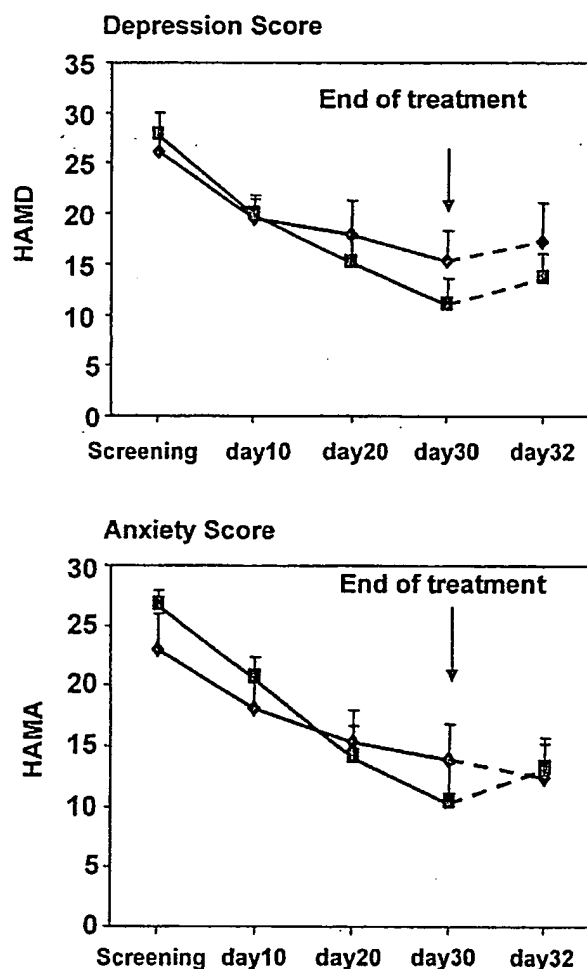


Fig. 3. Changes of HAMD and HAMA rating-scale scores in panel 1 (diamond) and panel 2 (square) show significant reductions in both panels and markedly better improvements in panel 2. In both panels HAMD rating-scale scores worsened after drug discontinuation. The HAMA rating-scale scores show increases after drug discontinuation in panel 2, but not in panel 1.

Table 2

Changes of rating-scales scores relative to screening during both panels. Note that absolute score differences (i.e. value at screening minus value at day of treatment) decreased more markedly in panel 2 than in panel 1. If the drop-out cases would have been included in panel 2 according to an intent-to-treat procedure, the HAMD mean \pm SEM (p) changes would have been: day 10: 6.8 ± 1.37 ($p < 0.001$); day 20: 9.5 ± 2.19 ($p = 0.001$); and day 30: 12.5 ± 2.36 ($p < 0.001$), suggesting that inclusion of the four drop-out cases would not have invalidated the overall conclusions

Panel 1 (<i>n</i> = 10)					Panel 2 (<i>n</i> = 10)			
Depression								
Time (days)	HAMD		BDI		HAMD		BDI	
	M ± SEM	<i>p</i>	M ± SEM	<i>p</i>	M ± SEM	<i>p</i>	M ± SEM	<i>p</i>
Screening								
—day 10	6.7 ± 2.07	0.010	1.5 ± 2.34	0.537	7.7 ± 1.52	0.001	6.6 ± 3.16	0.066
—day 20	8.3 ± 3.07	0.024	3.9 ± 2.86	0.206	12.4 ± 2.43	0.001	9.2 ± 4.23	0.058
—day 30	10.8 ± 2.72	0.003	6.0 ± 2.76	0.058	16.6 ± 2.05	0.001	14.3 ± 3.93	0.005
Anxiety								
	HAMA		STAI		HAMA		STAI	
Screening								
—day 10	4.9 ± 2.00	0.037	1.4 ± 1.71	0.435	6.1 ± 2.38	0.031	4.8 ± 2.63	0.101
—day 20	7.6 ± 2.59	0.017	1.3 ± 2.37	0.597	12.6 ± 3.04	0.003	7.2 ± 2.30	0.012
—day 30	9.1 ± 3.35	0.024	3.8 ± 2.57	0.174	16.5 ± 3.23	0.001	13.3 ± 3.17	0.002
Clinical global impression								
	CGI				CGI			
Screening								
—day 10	0.2 ± 0.20				0.5 ± 0.17			
—day 20	0.5 ± 0.34				1.2 ± 0.29			
—day 30	0.9 ± 0.31				1.9 ± 0.23			

suicidality, at day 20 in one case. These four cases were substituted and were not included in the analyses. They are described below and the impact of their exclusion upon the overall study outcome is discussed separately.

Fig. 3 illustrates the changes of the mean HAMD values over time and demonstrates that cessation of drug treatment resulted in a worsening of depressive symptomatology in 12 patients (six patients in each panel). These changes were independent from treatment outcome because worsening occurred among three nonresponders in panel 1 and three nonresponders in panel 2 (see Table 1). Similar trends emerged from analysis of the depression self-rating scores (see Table 2).

When anxiety symptoms were analysed separately significant improvements according to HAMA scores were again observed (Fig. 3). As summarized in Table 2, HAMA and STAI scores dropped in both panels, but these changes were more pronounced in panel 2, where following cessation of treatment,

anxiety symptoms also worsened. When comparing depression and anxiety ratings completed by the study clinicians (HAMD and HAMA) and by the patients (BDI and STAI), we found that at the time point of screening only depression-associated (HAMD and BDI) and anxiety-associated (HAMA and STAI) ratings seemed to be significantly correlated ($p < 0.05$), while construct-divergent correlations (HAMD/STAI or HAMA/BDI) were absent (see Table 3). These insignificant correlations reached significance ($p < 0.05$) at day 30, which is consistent with the treatment-related beneficial effects on both anxiety-related and depression-related symptoms. The differences of clini-

Table 3

Correlations of screening (baseline) anxiety and depression-related rating-scale scores according to Kendall's tau

	BDI	STAI
HAMD	0.34 ($p = 0.042$)	0.29 ($p = 0.083$)
HAMA	0.262 ($p = 0.117$)	0.391 ($p = 0.019$)

cian-rating scores compared with baseline (screening), started to reach the level of significance at day 10 (HAMD, HAMA) in both panels, while patient-rating scores improved significantly only in panel 2 at day 30 (BDI) and day 20 (STAI), indicating a faster decrease in panel 2 (see Table 2).

CGI scores also dropped significantly and here again the reduction of scores relative to the screening day was enhanced in panel 2, where significant improvements began to be noted at day 10. Similar effects emerged in panel 1, however, only at the end of the 30-day study (see Table 2).

3.5. Prediction of response

UFC values, plasma ACTH and cortisol concentrations at screening or during the study were not found to correlate with any measure of treatment outcome. However, the ACTH response measured at the first and second CRH test (days 9 and 19) was correlated with the HAMD and BDI response at the end of the study (see Table 4). At the end of the trial (day 29), when 13 patients had responded, such correlations were no longer found. Those patients who had a lower CRH-elicited ACTH response at the beginning of the study were more likely responders according to the HAMD or BDI criterion at the end of the study.

3.6. Drop-out cases

In order to test whether the differences observed between both panels (suggesting that the higher dose in panel 2 was superior to the lower dose in panel 1) were merely due to exclusion of four potentially nonresponding drop-out cases, we analysed the effect of these four drop-out cases according to an intention-to-treat analysis. This analysis used a last visit carry over approach, submitting that the data at the time point of study discharge would not have changed if the patients had been maintained. According to this conservative approach, the superiority of reductions in depression and anxiety severity in panel 2 is still present according to both clinicians' and patients' ratings, although the effects were less marked (see legend Table 2).

Table 4
Correlations (Spearman Rho) of Δ AUC values of CRH-stimulated net ACTH output with HAMD and BDI response scores at the end of the study

Δ AUC	HAMD response		BDI response	
	Spearman Rho	<i>p</i>	Spearman Rho	<i>p</i>
Post CRH-pre CRH				
ACTH: day 9	-0.46	0.04	-0.43	0.06
ACTH: day 19	-0.60	0.007	-0.62	0.005
ACTH: day 29	-0.19	0.42	-0.30	0.20

The UFC values also decreased moderately when drop-out cases were included and this reduction in UFC values was more pronounced in panel 2. The plasma ACTH and cortisol levels which were found to be unaffected by R121919 among those completing the study in both panels remained unaffected when the four drop-out cases were included into the analyses. In addition, inclusion of drop-out cases did not invalidate the conclusion that treatment with R121919 failed to impair HPA responsiveness to CRH stimulation. The observation that blunted ACTH responses to CRH stimulation was predictive for a favorable response to treatment with R121919 and that high ACTH release indicates a less-favorable outcome was also confirmed following inclusion of the four drop-out cases.

4. Discussion

The main purpose of this study was to test whether a drug that antagonizes CRH₁ receptors is safe and well tolerated when administered to patients suffering from major depression. The clinical monitoring of laboratory tests, including clinical chemistry, ECG and EEG, proved that administration of R121919 was safe under the dose range and during the time period tested. Moreover, the drug was very well tolerated as none of the patients reported any subjective adverse effects. The effect of R121919 on endocrine regulation was of particular interest, since in rodents blockade of CRH₁ receptors had reduced their corticotrophic responsiveness to CRH. This could be a problem whenever an acute stress-induced increase of ACTH and cortisol is needed as part of an individual's defense mechanism, e.g. in the case of inflammation (Webster et al., 1996). Acute hormonal stress response is mediated by the CRH produced and released from the hypothalamus and acting at corticotrophs. Because cognitive or physical stressors would have been inappropriate under the clinical condition, intravenous CRH stimulations were repeatedly administered at escalating dosages of R121919 and, as illustrated in Fig. 2, the CRH-elicited ACTH secretions were indistinguishable across all dosages ranging from 5 to 80 mg, strongly suggesting that a complete blockade of the peripheral stress hormone system was unlikely. This interpretation is supported by the comparisons of UFC values, which found that this measure of overall adrenocortical secretory activity decreased only to a minor extent during treatment in both panels. This change can be attributed to the clinical improvement known to be associated with decreased HPA drive rather than to drug-induced HPA impairment (Holsboer and Barden, 1996). In fact, the UFC values remained in the normal range at any time point tested. Similarly, no specific effects of R121919 upon the cir-

cadian rhythms of plasma ACTH and cortisol secretion was noted, rejecting the possibility that partial blockade of CRH₁ receptors may interfere with circadian changes of HPA activity. In accordance with UFC values which only slightly decreased during treatment with R121919 in both panels, the mean plasma cortisol levels pooled over all five samplings tended to decrease, which is in accord with well-established effects of antidepressants on HPA activity in major depression (Holsboer and Barden, 1996). The lack of suppression of ACTH and cortisol by a CRH₁ receptor antagonist is consistent with the view that basal corticotrophic activity is independent from CRH. This is supported in preclinical studies where mice lacking functional CRH₁ receptors have unchanged plasma ACTH and corticosterone levels at baseline (Timpl et al., 1998). When confronted with a physical stressor, however, or when exposed to CRH, the corticotrophic cells of mouse mutants without CRH₁ receptors and of rats treated with high dosages of R121919 have suppressed plasma ACTH and corticosterone secretions which contrasts with the findings reported here (Timpl et al., 1998; Keck et al., unpublished observations). Nonhuman primates, however, were found to have CRH₂ receptors in the pituitary (Sánchez et al., 1999), and if this is also true in humans, the releasability of ACTH following a CRH infusion suggests that in the case of partial or even complete blockade of CRH₁ receptors appropriate ACTH release may be achieved through corticotrophic CRH₂ receptors. Because decrease or absence of CRH₁ receptors in heterozygous or homozygous null-mutant mice is associated with increased production and release of vasopressin, an amplification of CRH effects upon ACTH release may occur (Müller et al., unpublished observation; de Goeij et al., 1991; Wotjak et al., 1996). Alternatively, the blockade of corticotrophic CRH₁ receptors by R121919 under the current study conditions could have been incomplete, leaving sufficient CRH₁ receptors available for adequate CRH-elicited hormonal effects. While this possibility can not be completely rejected it seems unlikely to be the case because the CRH-stimulated ACTH release remained unchanged over a dose range from 5 to 80 mg of R121919.

We were interested as to whether neuroendocrine laboratory markers would predict the response of depressive symptomatology to R121919. Theoretically, if CRH hypersecretion would constitute a phenomenon that is present at all levels of CRH regulation and signaling, one would predict that the patients with peripheral indicators for HPA overactivity would be more likely to respond to a CRH-receptor antagonist. In the current study, baseline HPA measures (UFC and plasma ACTH and cortisol levels) at screening or during drug treatment were not predictive of clinical outcome. Interest-

ingly, CRH-stimulated ACTH measured after initiation of treatment with R121919 corresponded with the decrease in the HAMD score at the end of trial. One attractive explanation would be that increased CRH release from the limbic brain decreased the number of CRH receptors at the anterior pituitary, which results in the decreased responsiveness observed when stimulated by exogenous CRH. Thus, CRH hypersecreting patients as identified by blunted ACTH response to a CRH challenge, would be expected to more likely benefit from a CRH-receptor-blocking drug than patients with normal CRH secretory activity, having normal ACTH responses in CRH tests. This interpretation, however, is hampered by the differences in CRH and CRH-receptor regulation throughout the brain. While the CRH synthesis is suppressed at the level of the hypothalamus by glucocorticoids, either indirectly through protein-protein interactions between activated glucocorticoid receptors and transcription factors or directly through negative response elements in the CRH gene promoter (Malkoski and Dorin, 1999), expression of CRH can also be enhanced by glucocorticoids in other brain areas (Schulkin et al., 1998). In the amygdala, or those hypothalamic nuclei which project to the spinal cord, evidence has been provided that CRH expression is not suppressed but rather increased by glucocorticoids (Swanson and Simmons, 1989; Schulkin et al., 1998). Thus, CRH may be indirectly enhanced in the amygdala and cerebrospinal fluid by hypercortisolism originating from those hypothalamic nuclei projecting to the median eminence. It is likely that other regulatory elements such as CRH-binding protein (Potter et al., 1994) and as yet to be identified CRH receptors, are also participating in CRH effects at various neural circuits in the CNS. These complex interactions indicate that a peripheral HPA measure (UFC or CRH test results) is not necessarily reflective of the level of CRH₁-receptor activation in those brain areas implicated in the neuropathology of depression.

In this study, a number of psychopathometric scales were also applied which collectively showed significant reductions in the severity of depression and anxiety. Because of the open-label nature of the study any conclusions related to antidepressant effects of R121919 are limited. We can not rule out the possibility that nonspecific, placebo-like effects and clinician biases might have accounted for the changes in depression severity observed in the patients. Several observations support the notion that the data collected in this study give reason to further explore the potential of CRH-receptor antagonists as psychotropic drugs in controlled studies:

1. The group in panel 2 receiving higher drug dosages had a better overall response than patients receiving the lower dose regimen. This dose/response relationship speaks against a placebo-like effect.
2. After discontinuation of the drug a worsening of symptomatology occurred, which is an unlikely event under clinical routine ('wash-out') conditions and favors the possibility of a specific drug effect. This view is supported by the observation that not only patients who responded favorably, but also those with poor or absent treatment effects showed worsening of depressive psychopathology (HAMD scores) when discontinued from R121919 (see Fig. 3)
3. The possibility of clinician's biases as a major confound also seems unlikely because results from the patient-rated inventories (BDI and STAI) matched those of the clinician-rated instruments (HAMD, HAMA and CGI).

At the time of screening, when patients were examined prior to treatment there were only correlations between depression-related instruments (HAMD and BDI) and between anxiety-related instruments (HAMA and STAI). Construct-divergent correlations, i.e. between HAMD and STAI or HAMA and BDI were absent at screening. However, at the end of the study all rating scores, especially their changes between screening and endpoint were strongly correlated, which agrees with a positive overall effect that can not be primarily attributed to a sole anxiolytic effect. If the latter were the case, the patient- and clinician-rated depression scores would have been correlated with the corresponding anxiety scores at enrollment.

In this open-label study we have found significant reductions in patient- and clinician-rated depression and anxiety scores. Comparing the degree of clinical improvement over subsequent clinical ratings it becomes apparent that these changes tended to be earlier and more pronounced among those patients who entered panel 2, receiving higher dosages. Because of this dose-response effect the high correlations between patients' and clinicians' rated changes in symptom scores, and the outcome of independent worsening of psychopathology after drug discontinuation, we believe that the observations reported here can not be attributed just to methodological issues. Of course, we are aware that the findings reported here are preliminary and that the purpose and design of the study allowed only descriptive analysis.

The clinically observed beneficial effects on depressive symptoms, as well as the absence of clinically relevant adverse effects, particularly interference with neuroendocrine regulation, justify validation of the postulated efficacy of R121919 in controlled trials. Another suggestion from the current study is the need for clinical tests that help to identify those patients

which are likely to respond to R121919 and other CRH₁-receptor antagonists in the future. It is important to note that the activity of CRH neurons does not change in a uniform way in the brain, and increased CRH signaling through CRH₁ receptors is not necessarily manifested by increased hypothalamic CRH secretion into portal vessels. Thus, measuring ACTH and cortisol in the periphery might only poorly predict clinical response to a CRH₁-receptor antagonist.

Another issue emerging from this and other studies trying to elaborate the therapeutic potential of drugs that work through new nonconventional mechanisms relates to diagnostic boundaries. CRH-receptor antagonists work through a mechanism that prevents the consequences of hyperexposure of neural substrates to CRH (Holsboer, 1999). The resulting excessive CRH-receptor signaling may have many reasons ranging from genetically impaired corticosteroid-receptor signaling that leads to impaired negative feedback upon CRH secretion to the sequelae of severe trauma, particularly during early childhood. The consequence of such genetic and/or acquired impairments of HPA regulation renders an individual likely to develop a psychiatric disease, particularly under conditions of stressful events. Thus, future studies exploring the therapeutic potential of CRH-receptor antagonists will encompass not only depression but also anxiety disorders, stress-related sleep disorders, anorexia, stress-induced psychoses and withdrawal from substance abuse.

Acknowledgements

Part of this study was funded by Janssen Research Foundation (GERI).

References

- Chalmers DT, Lovenberg TW, Grigoriadis DE, Behan DP, De Souza EB. Corticotropin-releasing factor receptors: from molecular biology to drug design. *Trends Pharmacol Sci* 1996;17:166–72.
- Chen R, Lewis KA, Perrin MH, Vale WW. Expression cloning of a human corticotropin-releasing factor receptor. *Proc Natl Acad Sci USA* 1993;90:8967–71.
- Coplan JD, Andrews MW, Rosenblum LA, Owens MJ, Friedman S, Gorman JM, Nemeroff CB. Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult non-human primates exposed to early-life stressors: implications for the pathophysiology of mood and anxiety disorders. *Proc Natl Acad Sci USA* 1996;93:1619–23.
- Davis M. The role of the amygdala in fear and anxiety. *Ann Rev Neurosci* 1992;15:353–75.
- De Goeij DCE, Kvetnansky R, Whitnall MH, Jezova D, Berkenbosch F, Tilders FJH. Repeated stress-induced activation of corticotropin-releasing factor neurons enhances vasopressin stores and colocalization with corticotropin-releasing factor in the median eminence of rats. *Neuroendocrinology* 1991;53:150–9.

- Gold PW, Chrousos G, Kellner C, Post R, Roy A, Augerinos P, Schulte H, Oldfield E, Loriaux DJ. Psychiatric implications of basic and clinical studies with corticotropin-releasing factor. *Am J Psychiatry* 1984;141:619–27.
- Grigoriadis DE, De Souza EB. Small molecule CRF₁ receptor antagonists: characterization and clinical application. In: *Neuroendocrine workshop on stress sponsored by the Am. Neuroendocrine Soc.*: June 21–23, 1998, New Orleans, LA, 1998. p. 36.
- Heinrichs SC, Lapansky J, Lovenberg TW, De Souza EB, Chalmers DT. Corticotropin-releasing factor CRF₁, but not CRF₂ receptors mediate anxiogenic-like behavior. *Regul Peptides* 1997;71:15–21.
- Holsboer F. Neuroendocrinology of mood disorders. In: Bloom FE, Kupfer DJ, editors. *Psychopharmacology: The fourth generation of progress*. New York: Raven Press, 1995. p. 957–69.
- Holsboer F. The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety. *J Psychiat Res* 1999;33:181–214.
- Holsboer F, Barden N. Antidepressants and HPA regulation. *Endocrine Rev* 1996;17:187–205.
- Holsboer F, von Bardeleben U, Gerken A, Stalla GK, Müller OA. Blunted corticotropin and normal cortisol response to human corticotropin-releasing factor in depression. *N Engl J Med* 1984;311:1127.
- Holsboer F, Gerken A, von Bardeleben U, Grimm W, Beyer H, Müller OA, Stalla GK. Human corticotropin-releasing hormone in depression. *Biol Psychiatry* 1986;21:601–11.
- Holsboer F, Spengler D, Heuser I. The role of corticotropin-releasing hormone in the pathogenesis of Cushing's disease, anorexia nervosa, alcoholism, affective disorders and dementia. *Prog Brain Res* 1992;93:385–417.
- Kalin NH. Biological effects of corticotropin-releasing hormone administered to rhesus monkeys. *Fed Proc* 1985;44:249–53.
- Keck FE, Holsboer F (2000): Hyperactivity of CRH neuronal circuits as a target for therapeutic interventions in affective disorders. *Peptides* (In press).
- Liebsch G, Landgraf R, Gersberger R, Probst JC, Wotjak CT, Engelmann M, Holsboer F, Montkowski A. Chronic infusion of a CRH₁ receptor antisense oligodeoxynucleotide into the central nucleus of the amygdala reduced anxiety-related behavior in socially defeated rats. *Regul Peptides* 1995;59:220–39.
- Liebsch G, Montkowski A, Holsboer F, Landgraf R. Behavioural profiles of two Wistar rat lines selectively bred for high or low anxiety-related behaviour. *Behav Brain Res* 1998;94:301–10.
- Liebsch G, Landgraf R, Engelmann M, Lörscher P, Holsboer F. Differential behavioural effects of chronic infusion of CRH₁ and CRH₂ receptor antisense oligonucleotides into the rat brain. *J Psychiat Res* 1999;33:153–63.
- Malkoski SP, Dorin RI. Composite glucocorticoid regulation at a functionally defined negative glucocorticoid response element of the human corticotropin-releasing hormone gene. *Mol Endocrinol* 1999;13:1629–44.
- Nemeroff CB, Widerlov E, Bissette G, Walleus H, Karlsson E, Eklund K, Kilts DC, Loosen PT, Vale W. Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* 1984;226:1342–4.
- Nemeroff CB, Owens MJ, Bissette G, Andorn AC, Stanley M. Reduced corticotropin-releasing factor binding sites in the frontal cortex of suicide victims. *Arch Gen Psychiatry* 1988;45:577–9.
- Owens MJ, Nemeroff CB. The physiology and pharmacology of corticotropin-releasing factor. *Pharmacol Rev* 1991;43:425–73.
- Plotsky PM. Pathways to the secretion of adrenocorticotropin: a view from the portal. *J Neuroendocrinol* 1991;3:1–9.
- Potter E, Sutton S, Conaldson C, Chen R, Perrin M, Lewis K, Sawchenko PE, Vale W. Distribution of corticotropin-releasing factor receptor mRNA expression in the rat brain and pituitary. *Proc Natl Acad Sci USA* 1994;91:8777–81.
- Raadsheer FC, Hoogendijk WJG, Stam FC, Tilders FJH, Swaab DF. Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. *Neuroendocrinology* 1994;60:433–6.
- Sánchez MM, Young LJ, Plotsky PM, Insel TR. Autoradiographic and in situ hybridization localization of corticotropin-releasing factor 1 and 2 receptors in nonhuman primate brain. *J Comp Neurol* 1999;408:365–77.
- Schulkin J, Gold PW, McEwen BS. Induction of corticotropin-releasing hormone gene expression by glucocorticoids: implication for understanding the states of fear and anxiety and allostatic load. *Psychoneuroendocrinology* 1998;23:219–43.
- Schulz DW, Mansbach RS, Sprouse J, Braselton JP, Collins J, Corman M, Dunaiskis A, Faraci S, Schmidt AW, Seeger T, Seymour P, Tingley 3rd FD, Winston EN, Chen YL, Heym J. CP-154,526: A potent and selective nonpeptide antagonist of corticotropin-releasing factor receptors. *Proc Natl Acad Sci USA* 1996;93:10477–82.
- Shaham Y, Erb S, Leung S, Buczek Y, Stewart J. CP-15,526, a selective, nonpeptide antagonist of the corticotropin-releasing factor₁ receptor attenuates stress-induced relapse to drug seeking in cocaine- and heroine-trained rats. *Psychopharmacology* 1998;137:184–90.
- Skutella T, Probst JC, Criswell H, Moy C, Breese G, Jirikowski GF, Holsboer F. Antisense oligodeoxynucleotide complementary to corticotropin-releasing hormone mRNA reduces anxiety in shuttlebox performance. *Neuroreport* 1994;5:2181–5.
- Skutella T, Probst JC, Renner U, Holsboer F, Behl C. Corticotropin-releasing hormone receptor (type 1) antisense targeting reduces anxiety. *Neuroscience* 1998;85:795–805.
- Smith GW, Aubry J-M, Dellu F, Contarino A, Bilezikjian IM, Gold LH, Hauser C, Bentley CA, Sawchenko PE, Koob GF, Vale W, Lee K-F. Corticotropin-releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. *Neuron* 1998;20:1093–102.
- Steckler T, Holsboer F. Corticotropin-releasing hormone receptor subtypes and emotion. *Biological Psychiatry* 1999;46:1480–508.
- Stenzel-Poore MP, Heinrichs SC, Rivest S, Koob GF, Vale WW. Overproduction of corticotropin-releasing factor in transgenic mice: a genetic model of anxiogenic behavior. *J Neurosci* 1994;14:2579–84.
- Swanson LW, Simmons DM. Differential steroid hormone and neural influences on peptide mRNA levels in CRH cells of the paraventricular nucleus: a hybridization histochemical study in the rat. *J Comp Neurol* 1989;285:415–35.
- Timpl P, Spanagel R, Sillaber I, Kresse A, Reul JMH, Stalla GK, Blanquet V, Steckler T, Holsboer F, Wurst W. Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. *Nature Genet* 1998;19:162–6.
- Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β -endorphin. *Science* 1981;213:1394–7.
- Valentino RJ, Foote SL, Page ME. The locus coeruleus as a site for integrating corticotropin-releasing factor and noradrenergic mediation of stress responses. *Ann NY Acad Sci* 1993;697:173–88.
- Webster EL, Lewis DB, Torpy DJ, Zachman EK, Rice KC, Chrousos GP. In vivo and in vitro characterization of antalarmin, a non-peptide corticotropin-releasing hormone (CRH) receptor antagonist: suppression of pituitary ACTH release and peripheral inflammation. *Endocrinology* 1996;137:5747–50.
- Wotjak CT, Kubota M, Liebsch G, Montkowski A, Holsboer F, Neumann I, Landgraf R. Release of vasopressin within the rat paraventricular nucleus in response to emotional stress: a novel mechanism of regulating adrenocorticotrophic hormone secretion? *J Neuroscience* 1996;16:7725–32.

Robertson, Ed.

oda, K. Koshiya, M.

acol.Exp.Ther., 283.

, P.A. Sargent, C.J.
R5 (1996).

arez, M. Marien, and

and J.L. Moreau,

rn, and U. Widmer,

nd J. Wichmann,

ot, Ed., Elsevier,

it, and U. Widmer,

Guardiola-Lemaitre,

i.L. Grassam, P.M.

wman, G. Riley, C.

, Jr., Drug News &

on, Br.J.Pharmacol.,

owski, E. Ber, G.G.

ull, N.M.J. Rupniak,

id.Chem., 41, 4607

amek, S.A. Reines,

oss, C.J. Swain, T.

, S. Sadowski, A.R.

d N.M.J. Rupniak,

joer, A. Pasini, and

ahir, and D. Beileli,

in, C.L. Kimbrough,

N. Gee, and M.B.

n, R.M. Woodward,

Q, 119 (1996).

Chapter 2. Recent Progress in Corticotropin-Releasing Factor Receptor Agents

James R. McCarthy*, Stephen C. Heinrichs† and Dimitri E. Grigoriadis
Neurocrine Biosciences Inc., San Diego, CA 92121-1102

*Lilly Corporate Center, Eli Lilly and Company, Indianapolis, IN 46285

†Psychology Dept., Boston College, Chestnut Hill, MA 02467

Introduction - Corticotropin-releasing factor (CRF) is a neurohormone which appears necessary and sufficient for the organism to mount functional, physiological and endocrine responses to stressors (1). Factors which mobilize brain CRF systems appear to have one feature in common, -the ability to disturb homeostasis. For example, demands on the organism may be induced either internally or externally by exposure to physical trauma, infection or social conflict. Coping responses to such deflections in steady state which include sympathetic nervous system activation, promotion of negative energy balance and augmentation of vigilance and emotionality appear to be CRF-dependent. Not surprisingly, many human psychopathologies which include hyperexcitability or anxiety-like components are hypothesized to depend either causally or symptomatically on overactivation of CRF in brain. This chapter will document recent progress towards the goal of assigning functional significance to endogenous CRF circuits in brain, and examine the state of the art regarding the growing array of pharmacological tools, including the most recently published small-molecule ligands, which are available for further probing the physiological significance of CRF system activation.

FUNCTIONAL SIGNIFICANCE OF CRF RECEPTORS AND CRF-BINDING PROTEIN

CRF Receptors - CRF receptors belong to the recently described family of "gut-brain" neuropeptide receptors. Other typical members of this family include receptors for calcitonin, vasoactive intestinal peptide, parathyroid hormone, secretin, pituitary adenylate cyclase-activating peptide, glucagon and growth hormone-releasing factor. All of these receptors possess seven putative transmembrane domains and are positively coupled to adenylyl cyclase.

The CRF₁ receptor was first cloned from several species including human (2, 3), mouse (3) and rat (4, 5). Species homologs are 98% identical over their full length of 415 amino acids. In general, the CRF₁ receptor is approximately 30% identical to all other members of the neuropeptide receptor family. Characteristic of most G-protein coupled receptors, the CRF₁ receptor has putative N-linked glycosylation sites on the N-terminal extracellular domain. There are five predicted sites on CRF₁, substantiating the glycosylation profiles determined by chemical affinity cross-linking studies (6). In addition, there are potential protein kinase C phosphorylation sites in the first and second intracellular loops and in the C-terminal tail, as well as casein kinase II and protein kinase A phosphorylation sites in the third intracellular loop (2).

There are currently three known forms of the CRF₂ receptor: CRF_{2a}, CRF_{2b} and CRF_{2r}. The CRF_{2a} receptor, which was originally described by Lovenberg et al. (7), is a 411 amino acid protein with approximately 71% identity to the CRF₁ receptor. The

CRF_{2b} receptor, which has been cloned from both rat (7) and mouse (8, 9), is 431 amino acids in length and differs from CRF_{2a} in that the first 34 amino acids in the N-terminal extracellular domain are replaced by 54 different amino acids. A third splice variant, the CRF_{2c} receptor, has recently been identified in human brain (10). This splice variant uses yet a different 5' alternative exon for its amino terminus and replaces the first 34 amino acid sequence of the CRF_{2a} receptor with a unique 20 amino acid sequence. RT-PCR analysis of human brain mRNA demonstrated expression in amygdala and hippocampus while southern analysis of rat genomic DNA yielded negative results, suggesting that this subtype does not exist in rat. All splice variants of the CRF₂ receptor have potential N-glycosylation and phosphorylation sites, which are analogous to those found in CRF₁ receptors. It is interesting to note that there are very large regions of amino acid identity between CRF₁ and CRF₂ receptors, particularly between transmembrane domain five and transmembrane domain six. This similarity argues strongly for conservation of biochemical function since it is this region which is thought to be the primary site of G-protein coupling and signal transduction.

CRF Binding-Protein (CRF-BP) - Plasma CRF is substantially elevated during the third trimester of human pregnancy and this process is likely to participate in a cascade of events which eventually leads to parturition (11). It was subsequently demonstrated that the majority of this late gestational maternal plasma CRF is bound to a high affinity CRF-binding protein (CRF-BP) which neutralizes the ability of CRF to release adrenocorticotrophic hormone (ACTH). Thus, the levels of CRF-BP in the maternal plasma determine the amount of 'free' CRF that is available to interact with pituitary CRF receptors and thereby modulate the activity of the pituitary-adrenocortical axis during late human pregnancy. The predominant tissues expressing CRF-BP in all species are the brain and the pituitary gland where the protein is hypothesized to regulate CRF actions (12). With respect to the CNS and the role of CRF-BP, it has recently been demonstrated that CRF-BP is expressed in various areas of brain including the cerebral cortex, amygdala, hippocampus, hypothalamus as well as sensory relays associated with the auditory, olfactory, vestibular and trigeminal systems. Of note, there are brain areas that are enriched with CRF and CRF-BP but have only low densities of receptors and conversely, other brain areas which are enriched with receptors and devoid of CRF-BP (13). Thus, the differential distribution of brain CRF-BP and CRF receptors presents multiple distinct sites of interaction with CRF for potential exploitation in the treatment of central deficits in CRF neurotransmitter function.

CRF Mutant Mouse Models - The profusion of CRF peptide, CRF post-synaptic receptor and CRF binding-protein transgenic and knockout mouse models reported over the past year allows for critical analysis of hypotheses relating pituitary-adrenocortical axis tone and brain CRF system activation in animal models to a variety of clinical psychopathologies. One phenotype common to all of the CRF mutants is altered tone/reactivity of the pituitary-adrenocortical axis. CRF overexpressor (transgenic) mice exhibit an overabundance of CRF in brain as well as 5-10 fold elevations in plasma levels of ACTH and corticosterone (14). CRF-knockout mice which are CRF deficient lose normal circadian variations in plasma ACTH and corticosterone which are restored by constant infusion of exogenous CRF (15). CRF₁

receptor knock attenuated ACTH (16). CRF binding stimulated plasma consequences in expression to accommodate at different secretory adrenocortical axis

The putative CRF₁ receptor knockout mice. The phenotype of CRF systems could be under- or over-expressed in flight response, knockdown, CRF-like attributes (17). CRF-BP which could have properties in knockout mice to modulate emotionality in the mediating human

CRF Gene Knockout - Oligonucleotide-mediated translational arrest is expected to be an effective administration of directed agents against vehicle-treated animals; the antisense-transcript procedure (20). CRF behaviors in pituitary-adrenocortical axis attenuates the effects. Consistent with the nucleus of the socially defeated receptors did not suggest that related behavior

Human Stress-Axis - Deficiency of CRF has been associated with consumption, diurnal behavioral characteristics and psychiatric disorders

ie (8, 9), is 431
acids in the N-
s. A third splice
rain (10). This
o terminus and
ith a unique 20
A demonstrated
of rat genomic
exist in rat. All
cosylation and
receptors. It is
identity between
lomain five and
conservation of
primary site of G-

d during the third
in a cascade of
ly demonstrated
bound to a high
f CRF to release
in the maternal
act with pituitary
renocortical axis
g CRF-BP in all
hypothesized to
f CRF-BP, it has
areas of brain
mus as well as
and trigeminal
and CRF-BP but
areas which are
ential distribution
of interaction with
deficits in CRF

RF post-synaptic
models reported
relating pituitary-
odels to a variety
: CRF mutants is
F overexpressor
well as 5-10 fold
F-knockout mice
isma ACTH and
CRF (15). CRF₁

receptor knockout mice display low basal levels of plasma corticosterone and attenuated ACTH and corticosterone stimulation in response to a restraint stressor (16). CRF binding protein transgenic mice which exhibit a normal pattern of basal and stimulated plasma ACTH and corticosterone levels appear to counterbalance the consequences of CRF binding protein enrichment via a compensatory 82% increase in expression of CRF (17). It is important to note that while the CRF mutants accommodate their genetic re-programming by adaptation of the endocrine stress axis at different set points, each mutant retains the ability to activate the pituitary-adrenocortical axis, albeit in diminished or altered fashion, in response to stress.

The putative role of brain CRF in affective disorders has been examined using CRF₁ receptor knockout mice and CRF binding-protein overexpressing and knockout mice. The phenotype of CRF mutants with under- or over-activation of endogenous CRF systems can now be compared to the expected consequences of stress-axis under- or overactivation predicted by clinical findings (18). For instance, CRF overexpressor mice exhibit an anxiogenic-like phenotype characteristic of a fight or flight response, whereas deactivation of brain CRF systems via CRF neuropeptide knockdown, CRF₁ receptor knockdown or knockout evokes complementary, anxiolytic-like attributes (19). Bionutralization of CRF by an indirect means, overexpression of CRF-BP which complexes and neutralizes endogenous CRF, also produces anxiolytic-like properties *in vivo*. Elucidation of affective phenotypes for CRF and CRF₂ receptor knockout mice will soon be possible. The positive correlation between CRF levels and emotionality in these animal models is in keeping with the hypothesized role of CRF in mediating human affective diseases.

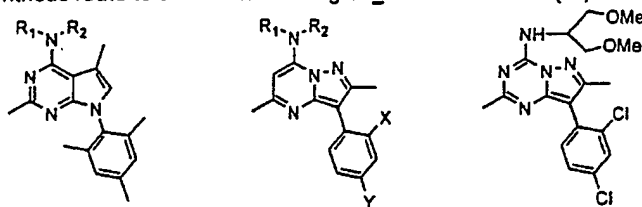
CRF Gene Knockdown - Gene knockdown, also referred to as antisense oligonucleotide targeting, suppresses expression of a particular gene product via translational arrest. Thus, the *in vivo* consequences of gene knockdown would be expected to be consistent with the pattern of functional effects of gene deletion or administration of competitive receptor antagonists. Treatment of rats with an antisense directed against CRF was shown to produce anxiolytic-like effects. Compared to vehicle-treated animals, lower ACTH and corticosterone levels were also observed in the antisense-treated animals following exposure to a shuttle-box avoidance procedure (20). Recent antisense studies focused on CRF₁ and anxiety-related behaviors in particular have demonstrated that treatment with CRF₁ antisense attenuates the anxiogenic-like effects induced by central administration of CRF (21). Consistent with this finding, chronic infusion of a CRF₁ antisense into the central nucleus of the amygdala was also reported to reduce anxiety-related behaviors in socially defeated rats (22). In contrast, treatment with antisense directed against CRF₂ receptors did not affect performance in the defensive withdrawal paradigm, thus suggesting that CRF₂ may not participate in the neural pathways mediating anxiety-related behaviors (23).

Human Stress-Axis Pathophysiology - In rodent animal models, central administration of CRF has been demonstrated to produce anxiety-like effects, decreased food consumption, diminished sexual behavior and altered sleep patterns (24). These behavioral changes closely parallel signs and symptoms observed in human psychiatric disorders including major depression, anxiety disorders and anorexia

nervosa, thus suggesting a role for CRF in the pathophysiology of mental illness. Accordingly, CRF hypersecretion has been detected in a large portion of individuals diagnosed with major depression (18). In depressed patients, elevated cortisol levels and a blunted ACTH response to CRF administration were observed, thus supporting the notion that the hypercortisolism observed in major depression is due to abnormal CRF secretion in the central nervous system. Noteworthy, treatment of depressed patients with antidepressant drugs or electroconvulsive therapy resulted in a decrease of the altered levels of CRF observed before the antidepressant treatment as well as in improvements in the clinical conditions of these patients. This observation strengthens the hypothesis that overactivation in brain CRF pathways may underlie certain features of the symptomatology seen in affective disorders, in which case CRF receptor antagonists would be expected to have a therapeutic benefit. In complementary fashion, CRF receptor agonist administration could conceivably counteract the consequences of diminished activation of brain CRF circuits (25).

CRF RECEPTOR ANTAGONISTS, AGONISTS AND CRF-BP LIGAND INHIBITORS

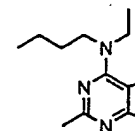
Non-peptide CRF₁ receptor antagonists that can selectively block the CRF₁ receptor subtype inhibit CRF-stimulation of cAMP or CRF-stimulated ACTH release from cultured rat anterior pituitary cells (26). Furthermore, when administered peripherally, these compounds compete for *ex vivo* [¹²⁵I]saugvine binding to CRF₁ receptors in brain sections demonstrating their ability to cross the blood-brain-barrier. Peripheral administration of these compounds attenuates stress-induced elevations in plasma ACTH levels demonstrating that CRF₁ receptors can be blocked in the periphery. Furthermore, peripherally administered CRF₁ receptor antagonists have also been demonstrated to inhibit CRF-induced seizure activity (27) suggesting that non-peptide CRF₁ receptor antagonists, when administered systemically, also specifically block central CRF₁ receptors. Extensive evidence validates the ability of CP-154,526 (**1**) to competitively antagonize CRF₁ receptors. This compound attenuates fear-potentiated startle after oral administration in rats (28) and in the same study the oral bioavailability of **1** was estimated to be 37%. Comparison of the inhibition of binding of 70 pM [¹²⁵I]Tyr⁰-o-CRF to rat brain by the monobutyl analog (**2**) (IC₅₀ = 110 nM) and monoethyl analog (**3**) (IC₅₀ = 620 nM) of **1** (IC₅₀ = 5.5 nM) was made. A synthetic route to a dinitrated analog of **1** is now available (28).



- 1:** R₁ = n-Bu, R₂ = Et **4:** R₁ = H, R₂ = CH(Et)₂, X = CH₃, Y = OCH₃
2: R₁ = n-Bu, R₂ = H **5:** R₁ = CH₂-c-Pr, R₂ = n-Pr, X = Y = Cl
3: R₁ = H, R₂ = Et **6:** R₁ = n-Pr, R₂ = CH₂CH₂OCH₃, X = Y = OCH₃

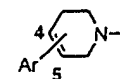
The efforts of several groups have focused on the synthesis of pyrazolo[1,5-a]pyrimidines as CRF receptor antagonists. After the publication of the first patent application (29), two additional world patent applications appeared (30, 31). The pharmacokinetics in dog of SP904 (**4**) (K_i = 1 nM) showed t_{1/2} (po) = 45 hours and oral

bioavailability of : potent analog, cyclopropylmethyl SAR study, NBI demonstrated effi 696, (7), (K_i = 1 pyrazolo[1,5-a]-s- t_{1/2} = 33.4 hours cardiovascular, C those effective in molecule improve



- 8:** R₁ = H
9: R₁ = CH₃
10: R₁ = CH₂-c-P

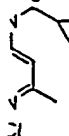
Certain N-7 imidazopyridine-2 small N-7 alkyl gr comparing **8** (K_i : the 8-oxo group while the 1-dea; d)pyrimidine thio substituted pher antagonists. CR₁ found to have (compounds to r activity amelior aminopyrimidine-41). DMP 695 (**1** receptor antagonist



- 14:** Ar :
15: Ar :

While a wide molecule CRF₁ r which has receiv may correlate w increase expone possibly serve a CRF levels were that CRF recep hypothesis, infus

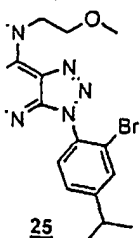
reported by the agonists.



1

it CRF receptor
AR study on the
nM) reported a

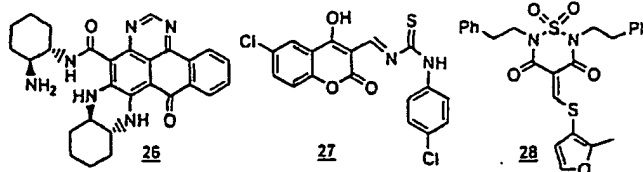
atic SAR studies
imilar to a model
s to rotation of
ods, X-ray and
ted the active
ditional anilino-
24) ($K_i = 32$ nM)
ral bioavailability
ours. However,
clic triazolo[4,5-
udy, SC241 (25)
ntial therapeutic
d (51).



25

(26), the number
here is a scarcity
re appeared that
eneric structures
le CRF receptor

olabeled o-CRF
Compound 26
 R_1 /CRF $_2$ receptor
ation and CRF-
g an antagonist.
ber of different
:00 nM), D_2 (K_i =
against the other



The first patent application for CRF binding protein ligand inhibitors claiming structure 27, as well as related compounds, has recently appeared (54). Rats were treated with 1 mg/kg (po) of 27 and were subjected to the Morris Water Maze test. A slight improved performance was reported over control rats after three days of acquisition training. In a second patent application (55), compound 28 and related structures, were claimed as CRF binding protein ligand inhibitors. No biological data appeared in the patent application.

CRF RECEPTOR AND CRF-BP SELECTIVE PHARMACOLOGICAL TOOLS

Construction of CRF Peptide Analogs – The native rat/human CRF(1-41) peptide affords a variety of opportunities to study CRF neurobiology by virtue of the fact that discrete and separate sequences of amino acids within the peptide chain subserve CRF receptor binding, receptor signaling and CRF-BP binding functions (56). For instance, the cyclization of shortened agonists of CRF at positions 30–33 to form a lactam ring (e.g. cyclo(30-33)[Ac-Leu⁸,D-Phe¹²,Nle²¹,Glu³⁰,Lys³³,Nle³⁸]hCRF(8-41) (or the equivalent positions 29-32 in sauvagine and urocortin) induces an α -helical constraint that stabilizes a bioactive conformation of the peptides. In addition, evidence was presented that the leucine/isoleucine residues at position 8 of CRF peptide agonists bears sole responsibility for activation of the receptor (57). Similarly, the tertiary structure of CRF necessary to bind its postsynaptic receptors is provided by the aminoterminal region of the peptide whereas amino acids near the carboxyterminus are necessary for binding CRF-BP (58). These inherent physicochemical properties allow endogenous CRF family agonists such as r/h CRF(1-41) and rat or human urocortin (1-40) to be modified forming peptides such as α -helical CRF(9-41) or d-Phe CRF(12-41) which are competitive receptor antagonists and peptides such as r/h CRF(6-33) which is a selective CRF-BP ligand inhibitor (Table 1). This same approach may also provide receptor selective agonist peptides (59) which can guide the discovery of small molecule agonists such as 18 and 19.

In Vivo Efficacy Profile of CRF Receptor and CRFBP Ligands – The differential binding profile for CRF family ligands exhibited in Table 1 is mirrored by distinct functional actions following administration in animal models. For example, the competitive CRF receptor antagonists, α -helical CRF (9-41) and d-Phe CRF (12-41) both block CRF $_1$ and CRF $_2$ receptors and yet are easily distinguished *in vivo* by the fact that α -helical CRF (9-41), but not d-Phe CRF (12-41), produces partial agonist actions (60) which may be due to affinity of α -helical CRF (9-41) for the CRF binding-protein. This supposition is supported by functionally selective *in vivo* effects of an indirect CRF agonist, the competitive CRF binding-protein ligand inhibitor peptide rat/human CRF (6-33), relative to the profile of a full post-synaptic CRF $_1$ /CRF $_2$ receptor agonist such as CRF itself (61). Similarly, the rank order in affinity of urocortin (CRF binding-protein > CRF $_2$ receptor > CRF $_1$ receptor) favors a functional dissociation from CRF itself which has a slightly different rank order profile (CRF binding protein > CRF $_1$ receptor > CRF $_2$ receptor). Consistent with this interpretation, the dissociation kinetics of CRF and urocortin from CRF binding-protein reveal an irreversible binding profile for urocortin (62) which could account, in part, for differential *in vivo* efficacy of urocortin versus CRF reported in some studies (63). Urocortin has also been examined in a variety of rodent behavioral and physiological assays of motor activity, exploratory inhibition, cardiovascular tone, malaise and food intake.

These paradigms were selected for their sensitivity to central nervous system and physiological activation states induced by exposure to environmental stressors and exogenous administration of CRF-like peptides such as CRF itself, sauvagine and urotensin. Anorexic actions of urocortin *in vivo* appear to be analogous to those of CRF, although urocortin has been reported to be the more potent of the two agonists in reducing food intake in some (63) but not all (64) studies. Thus, the precise neurobiological substrates for *in vivo* actions of CRF-family neuropeptides will likely follow extensive pharmacological studies employing CRF receptor subtype and CRF binding-protein selective ligands.

Table 1. Binding Profile of CRF Family Ligands

Ligand	CRF ₁	CRF ₂	CRF-BP
r/h CRF(1-41)	Yes	Yes	Yes
r/h urocortin (1-40)	Yes	Yes	Yes
r/h CRF(6-33)	No	No	Yes
Non-peptide CRF-BP	No	No	Yes
Ligand Inhibitor (27,28)			
o α -helical CRF(9-41)	Yes	Yes	Yes
r/h d-Phe CRF(12-41)	Yes	Yes	No
CP-154,526 (1)	Yes	No	No
Non-peptide CRF ₁ /CRF ₂	Yes	Yes	No
Receptor Antagonist (25)			

Summary - The pace of discovery for CRF receptor antagonists has substantially increased in the last two years as the interest in antagonists by the pharmaceutical industry has increased. As noted in the conclusions of the last report in this series (26), there are three key challenges in the area of CRF research. These include: 1) the discovery of CRF₂ subtype-selective agents to define the physiological roles of these sites; 2) the clinical evaluation of CRF₁-selective ligands and 3) the discovery of subtype-selective nonpeptide radioligands to better define the anatomical distribution of these receptors. Substantial progress has been made on all three fronts since there are now small molecule CRF₂ receptor antagonists, albeit with mixed CRF₁/CRF₂ activity, as well as results of ongoing clinical trials with selective non-peptide CRF₁ receptor antagonists that should be available in the near future.

References

1. A.V. Turnbull and C. Rivier, *Proc Soc Exp Biol Med*, **215**, 1 (1997).
2. R. Chen, K.A. Lewis, M.H. Perrin and W.W. Vale, *Proc. Natl. Acad. Sci. (USA)*, **90**, 8967 (1993).
3. N. Vita, P. Laurent, S. Lefort, P. Chalon, J.M. Lelias, M. Kaghad, F.G. Le, D. Caput and P. Ferrara, *Febs Letters*, **335**, 1 (1993).
4. C.P. Chang, R.I. Pearce, S. O'Connell and M.G. Rosenfeld, *Neuron*, **11**, 1187 (1993).
5. M.H. Perrin, C.J. Donaldson, R. Chen, K.A. Lewis and W.W. Vale, *Endocrinology*, **133**, 3058 (1993).
6. D.E. Grigoriadis and E.B. De Souza, *Endocrinology*, **125**, 1877 (1989).
7. T.W. Lovenberg, C.W. Liaw, D.E. Grigoriadis, W. Clevenger, D.T. Chalmers, E.B. De Souza and T. Oltersdorf, *Proc. Natl. Acad. Sci. (USA)*, **92**, 836 (1995).
8. T. Kishimoto, R.V. Pearce II, C.R. Lin and M.G. Rosenfeld, *Proc. Natl. Acad. Sci. (USA)*, **92**, 1108 (1995).
9. M. Perrin, C. Donaldson, R. Chen, A. Blount, T. Berggren, L. Bilezikjian, P. Sawchenko and W. Vale, *Proc. Natl. Acad. Sci. (USA)*, **92**, 2969 (1995).
10. W.A. Kostich, A. Chen, K. Sperle and B.L. Largent, *Mol. Endocrinol.*, **12**, 1077 (1998).

11. F. Petraglia, P. F. M. Stomati, D.A. (
12. C.G. Kemp, R.J. (
13. D.T. Chalmers, T. Pharmacol. Sci.,
14. M.P. Stenzel-Poo Y Acad Sci, **780**,
15. L. Muglia, L. Jaco
16. G.W. Smith, J.-M Marchuk, C. Hau Neuron, **20**, 1093
17. T.M. Ramesh, I.J Abstr., **24**, 505 (1
18. C.B. Nemeroff, B
19. S.C. Heinrichs, T
20. H.C. Wu, K.Y. Ch
21. T. Skutella, J.C. (1998).
22. G. Liebsch, R. L Holsboer and A. M
23. S.C. Heinrichs, J. Pept, **71**, 15 (199
24. A.J. Dunn and C. (
25. G.P. Chrousos, (
26. P.J. Gilligan, P.R (1997).
27. T.Z. Baram and C
28. Y.L. Chen, R.S. Dunaikis, W.S. F 1749 (1997).
29. C. Chen, T.R. We
30. A.G. Arvanitis and
31. Y.L. Chen, WO 91
32. P.J. Gilligan, C. E American Chemic
33. T. Capiris, D.J. MacKenzie, T.A. Chemistry Sympo
34. D.J. Wustrow, T MacKenzie, T.A. 8, 2067 (1998).
35. K. Wilcoxon, C. C Souza and J.R. I Chemistry, Anahei
36. L. He, P.J. Gillig; Shelton, M. Smith of Medicinal Cher
37. J.P. Beck, A.G. / Fitzgerald, R. Z Meeting, Las Vega
38. C. Chen, R. Dagr Moran, T.R. Web (1996).
39. P.E. Aldrich, A.G D.E. Grigoriadis, Wasserman, WO
40. Y.L. Chen, WO 91
41. J.R. McCarthy, J Huang, Z. Liu, Y.F
42. R. Bakhavatchal: WO 97/35539 (19

ous system and
tal stressors and
f, sauvagine and
gous to those of
the two agonists
hus, the precise
eptides will likely
ubtype and CRF

CRF-BP

Yes
Yes
Yes
Yes

Yes
No
No
No

has substantially
a pharmaceutical
ort in this series
These include: 1)
iological roles of
the discovery of
mical distribution
ree fronts since
beit with mixed
h selective non-
r future.

i. (USA), 90, 8967

e, D. Caput and P.

1187 (1993).

ndocrinology, 133,

ers, E.B. De Souza

Acad. Sci. (USA),

P. Sawchenko and

1077 (1998).

11. F. Petraglia, P. Florio, R. Gallo, C. Salvestroni, M. Lombardo, A.D. Genazzani, C. Di Carlo, M. Stomati, D.A. G and P.G. Artini, *Horm Res*, **45**, 187 (1996).
12. C.G. Kemp, R.J. Woods and P.J. Lowry, *Peptides*, **19**, 1119 (1998).
13. D.T. Chalmers, T.W. Lovenberg, D.E. Grigoriadis, D.P. Behan and E.B. De Souza, *Trends Pharmacol. Sci.*, **17**, 166 (1996).
14. M.P. Stenzel-Poore, J.E. Duncan, M.B. Rittenberg, A.C. Bakke and S.C. Heinrichs, *Ann N Y Acad Sci*, **780**, 36 (1996).
15. L. Muglia, L. Jacobson and J.A. Majzoub, *Ann N Y Acad Sci*, **780**, 49 (1996).
16. G.W. Smith, J.-M. Aubry, F. Dellu, A. Contarino, L.M. Bilezikjian, L.H. Gold, R. Chen, Y. Marchuk, C. Hauser, C.A. Bentley, P.E. Sawchenko, G.F. Koob, W. Vale and K.-F. Lee, *Neuron*, **20**, 1093 (1998).
17. T.M. Ramesh, I.J. Karolyi, M. Nakajima, S.A. Camper and A.F. Seasholtz, *Soc. Neurosci. Abstr.*, **24**, 505 (1998).
18. C.B. Nemeroff, *Biol. Psychiatry*, **44**, 517 (1998).
19. S.C. Heinrichs, *Trends Pharmacol. Sci.*, (in press), (1999).
20. H.C. Wu, K.Y. Chen, W.Y. Lee and E.H.Y. Lee, *Neuroscience*, **78**, 147 (1997).
21. T. Skutella, J.C. Probst, U. Renner, F. Holsboer and C. Behl, *Neuroscience*, **85**, 795 (1998).
22. G. Liebsch, R. Landgraf, R. Gerslberger, J.C. Probst, C.T. Woljak, M. Engelmann, F. Holsboer and A. Montkowski, *Regul. Peptides*, **59**, 229 (1995).
23. S.C. Heinrichs, J. Lapsansky, T.W. Lovenberg, E.B. De Souza and D.T. Chalmers, *Regul. Pept.*, **71**, 15 (1997).
24. A.J. Dunn and C.W. Berridge, *Br. Res. Rev.*, **15**, 71 (1990).
25. G.P. Chrousos, *Ann. New York Acad. Sci.*, **851**, 388 (1998).
26. P.J. Gilligan, P.R. Hartig, D.W. Robertson and R. Zaczek, *Ann. Rep. Med. Chem.*, **32**, 41 (1997).
27. T.Z. Baram and C.G. Hatalski, *Trends Neurol. Sci.*, **21**, 471 (1998).
28. Y.L. Chen, R.S. Mansbach, S.M. Winter, E. Brooks, J. Collins, M.L. Corman, A.R. Dunaikis, W.S. Faraci, R.J. Gallaschun, A. Schmidt and D.W. Schulz, *J. Med. Chem.*, **40**, 1749 (1997).
29. C. Chen, T.R. Webb, J.R. McCarthy and K.M. Wilcoxon, *WO 97/29109* (1997).
30. A.G. Arvanitis and R.J. Chorvat, *WO 98/03510* (1998).
31. Y.L. Chen, *WO 98/08847* (1998).
32. P.J. Gilligan, C. Baldauf, A. Cocuzza, D. Chidester, L. Fitzgerald, R. Zaczek and H. Shen, *American Chemical Society National Meeting*, Boston, MA, 1998, *MEDI 135*.
33. T. Capiaris, D.J. Wustrow, M.R. Rubin, J.A. Knobelsdorf, H. Akunne, M.D. Davis, R. MacKenzie, T.A. Pugsley, K.T. Zoski, T.G. Heffner and L.D. Wise, *26th National Medicinal Chemistry Symposium*, Omni Richmond Hotel, Richmond VA, 1998, *D*.
34. D.J. Wustrow, T. Capiaris, R. Rubin, J.A. Knobelsdorf, H. Akunne, M.D. Davis, R. MacKenzie, T.A. Pugsley, K.T. Zoski, T.G. Heffner and L.D. Wise, *Bioorg Med Chem Lett*, **8**, 2067 (1998).
35. K. Wilcoxon, C. Chen, C. Huang, M. Haddach, Y.-F. Xie, L. Wing, D.E. Grigoriadis, E.B. De Souza and J.R. McCarthy, *American Chemical Society Abstracts, Division of Medicinal Chemistry*, Anaheim, CA, 1999, *MEDI 02*.
36. L. He, P.J. Gilligan, R. Zaczek, L. Fitzgerald, N. Kalin, J. McElroy, J. Saye, H. Shen, S. Shelton, M. Smith, G. Trainor and P. Hartig, *American Chemical Society Abstracts, Division of Medicinal Chemistry*, Anaheim, CA, 1999, *MEDI 04*.
37. J.P. Beck, A.G. Arvanitis, A.J. Cocuzza, D.R. Chidester, M.A. Curry, J.T. Rescinito, L.W. Fitzgerald, R. Zaczek and J.C. Calabrese, *American Chemical Society National Meeting*, Las Vegas, NV, 1997, *MEDI 094*.
38. C. Chen, R. Dagnino Jr., E.B. De Souza, D.E. Grigoriadis, C.Q. Huang, K.I. Kim, Z. Lui, T. Moran, T.R. Webb, J.P. Whitten, Y.F. Xie and J.R. McCarthy, *J. Med. Chem.*, **39**, 4358 (1996).
39. P.E. Aldrich, A.G. Arvanitis, R.S. Cheeseman, R.J. Chorvat, T.E. Christos, P.J. Gilligan, D.E. Grigoriadis, C.N. Hodge, P.J. Krenitsky, E.L. Scholfield, S.W. Tam and Z.R. Wasserman, *WO 95/10506* (1995).
40. Y.L. Chen, *WO 95/33750* (1995).
41. J.R. McCarthy, J.P. Whitten, T.R. Webb, J.Y. Ramphal, D.E. Grigoriadis, C. Chen, C.Q. Huang, Z. Liu, Y.F. Xie and R. Dagnino, *WO 96/39400* (1996).
42. R. Bakthavatchalam, A.G. Arvanitis, J.P. Beck, G.A. Cain, R.J. Chorvat and P.J. Gilligan, *WO 97/35539* (1997).

43. R. Bakthavatchalam, A.G. Arvanitis, P.J. Gilligan, R.E. Olson, D.W. Robertson, G.L. Trainor, S.C. Smith, L.W. Fitzgerald, R. Zaczek, H. Shen and D.D. Christ. 216 ACS National Meeting, Boston, MA, 1998, MEDI 134
44. J.R. McCarthy, S.C. Heinrichs and D.E. Grigoriadis, *Current Pharmaceutical Design*, **5**, 247 (1999).
45. M. Mclean, A. Bisits, J. Davies, R. Woods, P. Lowry and R. Smith, *Nature Medicine*, **1**, 460 (1995).
46. R. Smith, *Scientific American*, *March*, 68 (1999).
47. J.P. Beck, P. Tivitmahaisoon, B.K. Folmer, M.A. Curry, L.W. Fitzgerald, P.J. Gilligan, R. Zaczek, D.W. Robertson and W. Marshal. American Chemical Society Abstracts, Division of Medicinal Chemistry, 1999, MEDI 01
48. C.Q. Huang, M. Haddach, C. Chen, K.W. Wilcoxon, Y.-F. Xie, D. Grigoriadis, E.B. De Souza and J.R. McCarthy. American Chemical Society, Division of Medicinal Chemistry, Anaheim, CA, 1999, MEDI 003
49. C.N. Hodge, P.E. Aldrich, Z.R. Wasserman, C.H. Fernandez, G.A. Nemeth, A. Arvanitis, R.S. Cheeseman, R.J. Chorvat, E. Ciganek, T.E. Christos, P.J. Gilligan, P. Krenitsky, E. Scholfield and P. Strucely, *J Med Chem*, **42**, 819 (1999).
50. A.G. Arvanitis, P.J. Gilligan, R.J. Chorvat, R.S. Cheeseman, T.E. Christos, R. Bakthavatchalam, J.P. Beck, A.J. Cocuzza, F.W. Hobbs, R.G. Wilde, C. Arnold, D. Chidester, M. Curry, L. He, A. Hollis, J. Klaczkiwicz, P.J. Krenitsky, J.P. Rescinito, E. Scholfield, S. Culp, E.B. De Souza, L. Fitzgerald, D. Grigoriadis, S.W. Tam, Y.N. Wong, S.M. Huang and H.L. Shen, *J Med Chem*, **42**, 805 (1999).
51. R.J. Chorvat, R. Bakthavatchalam, J.P. Beck, P.J. Gilligan, R.G. Wilde, A.J. Cocuzza, F.W. Hobbs, R.S. Cheeseman, M. Curry, J.P. Rescinito, P. Krenitsky, D. Chidester, J.A. Yarem, J.D. Klaczkiwicz, C.N. Hodge, P.E. Aldrich, Z.R. Wasserman, C.H. Fernandez, R. Zaczek, L.W. Fitzgerald, S.M. Huang, H.L. Shen, Y.N. Wong, B.M. Chien, C.Y. Quon and A. Arvanitis, *J Med Chem*, **42**, 833 (1999).
52. T.E. Christos and A. Arvanitis, *Expert Opinion in Therapeutic Patents*, **8**, 143 (1998).
53. D.R. Luthin, A.K. Rabinovich, D.R. Bhuralkar, K.L. Youngblood, R.A. Bychowski, D.S. Dhanoa and J.M. May, *Bioorganic & Medicinal Chemistry Letters*, **9**, 765 (1999).
54. J.P. Whitten, J.R. McCarthy, Z. Liu, C.Q. Huang, P.E. Erickson, D.P. Behan, Y.F. Xie and R.F. Lowe, WO 97/45421 (1997)
55. C.H. Mitch and S.J. Quimby, WO 98/51312 (1998)
56. J. Rivier, C. Rivier, R. Galyean, A. Miranda, C. Miller, A.G. Craig, G. Yamamoto, M. Brown and W. Vale, *J. Med. Chem.*, **36**, 2851 (1993).
57. J. Rivier, S.L. Lahrachi, J. Gulyas, J. Erchevgyi, S.C. Koerber, A.G. Craig, A. Corrigan, C. Rivier and W. Vale, *J Med Chem*, **41**, 2614 (1998).
58. S.W. Sutton, D.P. Behan, L.L. Sabine, R. Kaiser, A. Corrigan, P. Lowry, E. Potter, M.H. Perrin, J. Rivier and W. Vale, *Endocrinology*, **136**, 1097 (1995).
59. E.T. Wei, H.A. Thomas, H.C. Christian, J.C. Buckingham and T. Kishimoto, *Peptides*, **19**, 1183 (1998).
60. F. Menzaghi, R.L. Howard, S.C. Heinrichs, W. Vale, J. Rivier and G.F. Koob, *J. Pharmacol. Exp. Ther.*, **269**, 564 (1994).
61. S.C. Heinrichs, E.A. Vale, J. Lapsansky, D.P. Behan, L.V. McClure, N. Ling, E.B. De Souza and G. Schulteis, *Peptides*, **18**, 711 (1997).
62. A. Ardali, J. Gottowik, S. Henriot, R.G. Clerc and G.J. Kilpatrick, *J. Neurosci. Meth.*, **80**, 99 (1998).
63. M. Spina, E. Merlo-Pich, R.K. Chan, A.M. Basso, J. Rivier, W. Vale and G.F. Koob, *Science*, **273**, 1561 (1996).
64. D.N. Jones, R. Kortikaas, P.D. Slade, D.N. Middlemiss and J.J. Hagan, *Psychopharmacol.*, **138**, 124 (1998).

Introduction - is the deposit the walls of c the cleavage c to self-aggreg pathogenesis three familial mutations, all for AD, which fibrillized A β overexpressin approach for l peptide. For c that blocking Furthermore, form A β , have binding to the in AD pathop sulfate prote aggregation is the symptoms techniques fo amyloid inhib update on the

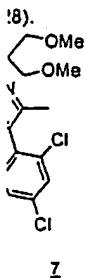
Bi

Rationale - Is useful to con occurs along a relatively s structure acti intermediate the activity is intermediate protein foldin a different s Different inhib vary. Therefo in the patholo fibrillization ir

of mental illness. ion of individuals ed cortisol levels l, thus supporting due to abnormal ent of depressed led in a decrease atment as well as This observation ays may underlie , which case CRF tic benefit. In ould conceivably ircuits (25).

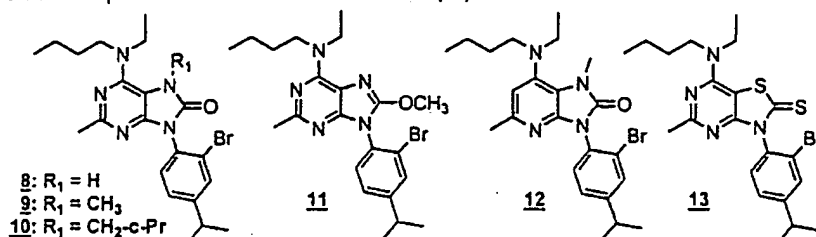
ND INHIBITORS

block the CRF₁ and ACTH release ren administered binding to CRF₁ ood-brain-barrier. iced elevations in : blocked in the antagonists have) suggesting that ystemically, also ates the ability of This compound) and in the same mparison of the obutyl analog (2) $IC_{50} = 5.5$ nM) was

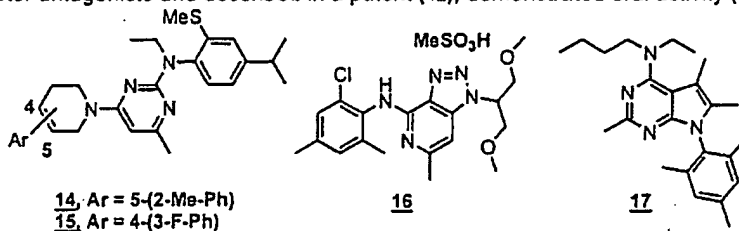


of pyrazolo[1,5-a] of the first patent ed (30, 31). The 45 hours and oral

bioavailability of 33.1 % (32). A SAR study of similar compounds found the most potent analog, **5**, to contain a 2,4-dichlorophenyl ring and a N-propyl N-cyclopropylmethyl amino side chain at the 7-position ($K_i = 3.2$ nM) (33, 34). From a SAR study, NBI 30545 (**6**) was selected for further study ($K_i = 2.8$ nM) and demonstrated efficacy in the CRF-induced locomotor activity model in mice (35). DMP-696, (**7**), ($K_i = 1.7$ nM) an orally active CRF receptor antagonist from the related pyrazolo[1,5-a]-s-triazine series (30), demonstrated 50% oral bioavailability in dogs with $t_{1/2} = 33.4$ hours and $t_{1/2} = 15$ hours in rhesus monkeys (p.o.). No endocrine, cardiovascular, GI, or pulmonary effects were noted at doses 30-fold higher than those effective in the rat situational anxiety assay. Furthermore, the potency of the molecule improves on chronic administration (36).

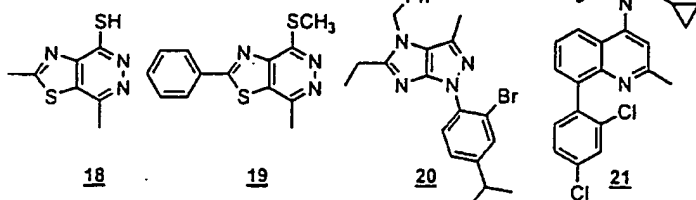


Certain N-7-alkyl-N-9-aryl-8-oxopurines, as well as structurally related imidazopyridine-2-ones, are CRF₁ receptor antagonists (37). The importance of a small N-7 alkyl group for tight binding of 8-oxopurines to the CRF₁ receptor is seen by comparing **8** ($K_i = 890$ nM), **9** ($K_i = 4.9$ nM) and **10** ($K_i = 117$ nM). O-Methylation of the 8-oxo group on **8**, to yield **11**, provides a more potent antagonist, ($K_i = 1.5$ nM) while the 1-deaza analog **12** ($K_i = 4.0$ nM) was equipotent to **9**. Thiazolo[4,5-d]pyrimidine thione **13** ($K_i = 4.1$ nM) contains the favored 2-bromo-4-isopropyl substituted phenyl ring common to a number of tight binding CRF receptor antagonists. CRA0165 (**14**) ($IC_{50} = 12.7$ nM) and CRA1000 (**15**) ($IC_{50} = 10.5$ nM) were found to have good affinity for the CRF₁ receptor. Oral administration of these compounds to mice and rats with stress-induced and CRF-induced anxiogenic-like activity ameliorated these effects in the 0.1 to 10 mg/kg range. Several anilino-aminopyrimidine-based CRF receptor antagonists have previously been reported (38-41). DMP 695 (**16**), obtained from the N-aryl aminotriazolopyrimidine series of CRF receptor antagonists and described in a patent (42), demonstrated oral activity (43).



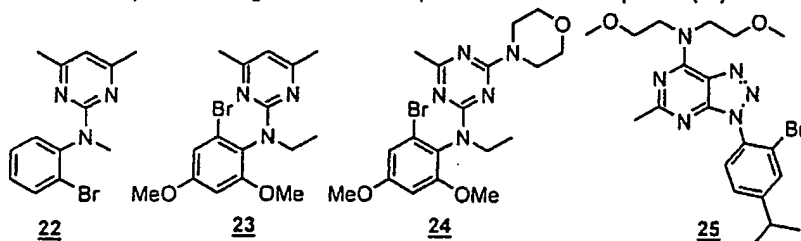
While a wide variety of anti-stress, anxiolytic and anti-depressant actions of small molecule CRF₁ receptor antagonists have been recently reported (44), one example which has received much attention concerns the fact that regulation of CRF levels may correlate with the length of pregnancy (45). In particular, plasma CRF levels increase exponentially as gestation advances beyond about 16 weeks and could possibly serve as a good predictor of delivery. Noting that women with the highest CRF levels were most likely to deliver prematurely, these investigators have posulated that CRF receptor blockade would act to delay parturition. In support of this hypothesis, infusion of antalarmin (**17**) to pregnant sheep delays delivery of lambs

until infusion is discontinued (46). Thiazolopyridazines **18** and **19** were reported by the same group to be promising leads in the development of CRF receptor agonists.



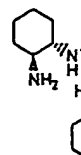
The imidazo[4,5-c]pyrazole **20** ($K_i = 4$ nM) is the most potent CRF receptor antagonist in a SAR study around this 5-5 bicyclic series (47). A SAR study on the contrasting 6-6 bicyclic 8-arylquinolines, represented by **21**, ($K_i = 0.5$ nM) reported a number of analogs with K_i values under 1 nM (48).

The screening lead **22** ($K_i = 5700$ nM) was optimized by systematic SAR studies that assisted in the definition of a pharmacophore (49) that is similar to a model previously proposed (38). Conformational preferences and barriers to rotation of anilino-aminopyrimidine **23** were determined by semiempirical methods, X-ray and variable temperature NMR spectroscopy. The study determined the active conformation of the anilino-pyrimidines and led to the synthesis of additional anilino-amino-based pyrimidine CRF receptor antagonists as well as SA627 (**24**) ($K_i = 32$ nM) (50). Compound **24** was evaluated in dog at 5 mg/kg (iv, po). The oral bioavailability was 20 % and the mean peak oral plasma level was 730 nM at 0.5 hours. However, this compound was not advanced further since members of the bicyclic triazolo[4,5-d]pyrimidine series were more promising. From an extensive SAR study, SC241 (**25**) ($K_i = 3.7$ nM) was believed to have properties necessary for a potential therapeutic agent. Further pharmacological studies are planned for this compound (51).



In the past two years, since the last review on CRF in this series (26), the number of patents and applications combined has nearly doubled. However, there is a scarcity of receptor binding data published in these patents. Two reviews have appeared that include these patents (44, 52). The more recent review summarizes generic structures and gives examples of structures claimed for all the small molecule CRF receptor antagonist patents (44).

Oxo-7H-benzo[e]spermidine-4-carboxamide **26** displaced radiolabeled α -CRF from both CRF₁ ($K_i = 110$ nM) and CRF₂ ($K_i = 20$ nM) receptors (53). Compound **26** is the most potent analog in a series of the first reported mixed CRF₁/CRF₂ receptor antagonists. The compound antagonized CRF-stimulated cAMP formation and CRF-stimulated corticotropin release from rat pituitary *in vivo*, suggesting an antagonist. The binding selectivity profile was determined for **26** at a number of different receptors. The compound showed weak binding to NPY Y1 ($K_i = 4200$ nM), D₂ ($K_i = 3200$ nM) and 5-HT₇ ($K_i = 1600$ nM), but binding $> 10,000$ nM against the other receptors screened.



The first patent structure **27**, as treated with 1 mg, slight improved acquisition training structures, were appeared in the

CRF RECEPTOR

Construction of a variety of discrete and specific CRF receptor binding site, for instance, the cyclic lactam ring (e.g., the equivalent of a constraint that evidence was provided by peptide agonists the tertiary structure by the amino terminal carboxy terminus physicochemical properties (41) and rat or human CRF(9-41) and peptides such as (Table 1). This is (59) which can give

In Vivo Efficacy
binding profile of functional action competitive CRF both block CRF₁ fact that α -helical actions (60) which protein. This is indirect CRF agonist rat/human CRF receptor agonist urocortin (CRF) dissociation from binding protein > the dissociation irreversible binding *in vivo* efficacy also been examined motor activity, e

BRESR 90109

Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses?

Adrian J. Dunn and Craig W. Berridge*

*Department of Pharmacology and Therapeutics, Louisiana State University Medical Center, Shreveport,
LA 71130–3932 (U.S.A.)*

(Accepted 27 March 1990)

Key words: Anxiety; Behavior; Corticotropin-releasing factor; Neurochemistry; Neuroendocrine; Norepinephrine; Stress

CONTENTS

1. Introduction	72
2. The cerebral distribution of CRF and binding sites for CRF	73
2.1. The cerebral distribution of CRF	73
2.2. The cerebral distribution of binding sites for CRF	73
3. Neurochemistry of endogenous and administered CRF	73
3.1. Release of cerebral CRF	73
3.2. CRF receptors	74
3.3. Neurochemical responses to CRF administration	75
4. Physiological effects of administered CRF	75
4.1. Endocrine effects of administered CRF	75
4.2. Autonomic effects of administered CRF	76
4.3. Electrophysiological effects of administered CRF	77
4.4. Gastrointestinal effects of administered CRF	77
4.4.1. Gastric acid secretion	77
4.4.2. Gastric emptying	78
4.4.3. Gastrointestinal motility	78
4.4.4. Gastric ulceration	78
5. Behavioral responses to administered CRF	79
5.1. Locomotor activity	79
5.2. Ingestive behavior	79
5.3. Antinociception	79
5.4. Grooming	80
5.5. Conflict tests and other tests of anxiety	80
5.5.1. Geller–Seifter conflict test	80
5.5.2. Social interaction	80
5.5.3. Acoustic startle	81
5.5.4. Elevated plus maze	81
5.5.5. Defensive withdrawal	81
5.5.6. Porsolt swim test	81
5.6. Conditioned emotional responses	81
5.7. Exploratory behavior	81
5.8. Shock-induced behaviors	81
5.8.1. Shock-induced freezing	81
5.8.2. Shock-induced fighting	83
5.9. Sexual behavior	83
5.10. Conditioned avoidance responding	83

* Present address: Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06510, U.S.A.
Correspondence: A.J. Dunn, Department of Pharmacology and Therapeutics, Louisiana State University Medical Center, P.O. Box 33932, Shreveport, LA 71130–3932, U.S.A.

5.11. Primate behaviors	84
6. CRF and the immune system	84
7. Involvement of the HPA axis in the effects of CRF	86
8. Involvement of the autonomic nervous system in the effects of CRF	86
9. Sites of action of CRF within the brain	87
10. Interaction of CRF with other neurotransmitter systems	88
10.1. Catecholamines	88
10.2. Serotonin	90
10.3. Acetylcholine	90
10.4. GABA	90
10.5. Endogenous opiates	90
11. The role of CRF in stress-related effects	91
12. Conclusions	91
12.1. The effects of CRF dose	92
12.2. Clinical relevance	93
Acknowledgements	93
References	93

1. INTRODUCTION

Harris¹⁰⁸ first postulated the existence of hypothalamic hormones that could be released from the median eminence of the hypothalamus to trigger the secretion of hormones from the adenohypophysis. These 'hypophysiotropic hormones' are more commonly known as releasing factors because of their critical role in releasing adenohypophyseal hormones. In 1955, Saffran and Schally²⁰¹ and Guillemin and Rosenberg¹⁰², independently provided the first convincing demonstrations of the existence of a factor derived from the hypothalamus that could elicit adrenocorticotropin (ACTH) secretion from the pituitaries of intact rats (for reviews, see Refs. 202 and 267). The factor was named corticotropin-releasing factor (CRF) because of its ability to stimulate secretion of ACTH. Although CRF was the first of the hypothalamic-releasing factors to be named, the elucidation of its structure was fraught with exceptional technical difficulties^{202,267} and the structures of several other hypothalamic releasing factors were determined earlier. Finally in 1981, Vale and his colleagues succeeded in determining the structure of ovine CRF (oCRF)²⁴⁶. Jean Rivier synthesized the compound according to the postulated structure and Vale's group showed that it had biological activity identical to that of the purified natural hormone²⁴⁶. The sequence of the hormone has now been determined in sheep, man, rats, pigs, goats and cows, and in all cases contains 41 amino acids and a similar primary structure (for a review, see Ref. 187). There is now little, if any, dispute that the 41-amino acid polypeptide synthesized by Vale et al. is the major endogenous

corticotropin-releasing factor, although a number of other factors (e.g. vasopressin (VP), catecholamines) are known to participate in the regulation of ACTH release^{8, 80,187}.

The availability of purified synthetic CRF and antibodies to it has enabled study of the biological activities of CRF. Histological studies with CRF antisera have indicated that CRF-like immunoreactivity (irCRF) exists in neurons outside the hypothalamus and that hypothalamic neurons send axons to regions of the brain other than the median eminence region^{208,209}. In addition, binding sites for CRF have been found in a distribution similar to irCRF. Neurochemical studies have indicated a Ca^{2+} -dependent release of CRF stimulated by K^+ (Ref. 224). Moreover, CRF has been shown to stimulate adenylate cyclase activity in slices of brain tissue, just as it does in the pituitary^{2,48,244,262}. Electrophysiological studies have shown regionally specific responses to iontophoretically applied CRF⁷⁶. Together, these results provide a strong basis for postulating a neurotransmitter role for brain CRF.

Administration of CRF to animals has indicated that the molecule has a variety of endocrine, physiological, neurochemical and behavioral activities that are not shared with ACTH or corticosterone. These observations have suggested a role for CRF beyond that of the regulation of ACTH release. Investigators in La Jolla (including Vale, Rivier, Brown, Fisher, Swanson and Koob) recognized that many of the effects of CRF resembled those observed in stress, suggesting the possibility that CRF may be an endogenous mediator of such responses¹³³. The purpose of this review is to survey the

rep
cer
hyp
we
in :
2. :
SIT
bra
204.
dis
ma
2.1
?
(P
me
mo
mi
Ho
bin
lan
fro
Ide
inv
anc
me
cell
a si
the
fini
niq
usi
by
spe
to
I
sys
ner
ing
Wi
cor
anc
mo
cor
II-
Otl
of i
olf
nig
coe

reports of administered CRF and to assess what role(s) cerebral CRF may play beyond that of an initiator of the hypothalamic-pituitary-adrenal (HPA) axis. Specifically, we shall examine the evidence for a role of cerebral CRF in stress.

2. THE CEREBRAL DISTRIBUTION OF CRF AND BINDING SITES FOR CRF

The distribution of CRF and CRF-binding sites in the brain has been described and reviewed by others^{158, 204, 231}. Although it is beyond the scope of this review to discuss this distribution in detail, a brief summary of the major findings will be presented.

2.1. The cerebral distribution of CRF

The paraventricular nucleus of the hypothalamus (PVN) is the primary source of CRF released from the median eminence into the portal blood supply, although most hypothalamic nuclei contain some irCRF and send minor projections to the median eminence^{158, 209, 231}. However, CRF-like material and specific, high-affinity binding sites have been identified in many extrahypothalamic regions of the CNS. A few CRF-positive fibers from the PVN project to brain stem autonomic nuclei²⁰⁸. Identification of CRF-like material within the CNS has involved both immunohistochemical methods^{158, 161, 231} and the combined use of HPLC purification and bioassay measuring the secretion of ACTH from anterior pituitary cells *in vitro*¹⁶⁶. Although both techniques have indicated a similar pattern of localization of CRF within the brain, the bioassay has not been used to study localization in as fine detail as that provided by immunostaining techniques. The importance of verifying CRF localization using techniques other than those based on recognition by antisera or at least using antisera with different specificities, is emphasized by a report that an antiserum to CRF also recognized substance P (ref. 15).

In general, irCRF is found in areas of the limbic system, structures involved in regulating the autonomic nervous system and regions associated with the processing of sensory information in both rats and primates. Within the telencephalon, the greatest number of irCRF-containing cell bodies was found in the prefrontal, insular and cingulate cortices^{158, 231}. A third study observed a more uniform distribution within the cortex²⁰⁴. IrCRF-containing perikarya were localized primarily to layers II-III with processes extending through layers I-IV. Other regions that contain relatively high concentrations of irCRF include the central nucleus of the amygdala, the olfactory bulb, certain thalamic nuclei, the substantia nigra pars compacta, the periaqueductal grey, the locus coeruleus (LC), the nucleus of the solitary tract, the

dorsal and ventral parabrachial nuclei and the cortex and deep cerebellar nuclei of the cerebellum^{86, 158, 204, 208, 231}. The relative concentration of irCRF detected in different brain structures varies in the different studies. This is probably accounted for by differences in the species studied, the antisera used and whether or not the animals were pretreated with colchicine.

Although the projection patterns of irCRF-containing neurons have not been described in detail, projections from the amygdala to the parabrachial nucleus¹⁶¹, from the inferior olivary nucleus to the cerebellum^{46, 56, 186} and from the perifornical and anterior hypothalamic areas to the lateral septum²⁰⁵ have been observed. The localization of CRF within limbic and autonomic structures provides an anatomical basis for the participation of CRF in coordinating visceral and behavioral responding.

2.2. The cerebral distribution of binding sites for CRF

Using both quantitative autoradiography and cell-membrane binding techniques, high-affinity binding sites for CRF have been observed in a pattern similar to that observed for irCRF^{63, 262}. A high density of CRF-binding sites was observed throughout neocortex with layers I and IV containing the greatest density of sites. Outside the CNS, specific CRF-binding sites have been located in all sympathetic ganglia, chromaffin tissue of the adrenal medulla and in other organs of the body, including the gut, pancreas and spleen²⁴⁴.

3. NEUROCHEMISTRY OF ENDOGENOUS AND ADMINISTERED CRF

3.1. Release of cerebral CRF

Evidence for a neurotransmitter role for CRF in the brain is provided in part by the anatomical evidence discussed above for parallel distributions of immunoreactive and bioactive CRF and of CRF-binding sites. In addition, there is evidence for a neurotransmitter-like release of CRF from brain tissue. Suda et al. observed release of irCRF from perfused rat hypothalami *in vitro*²²⁹. The release induced by K⁺ was completely dependent on the presence of Ca²⁺ in the incubation medium. Subsequently, Smith et al. demonstrated a similar phenomenon for extrahypothalamic regions of the brain²²⁴. Minced brain tissue from the amygdala, mid-brain, and striatum, like that from hypothalamus, was shown to release irCRF following stimulation with 56 mM K⁺ or scorpion venom, in a manner requiring Ca²⁺. No such effect was observed with cerebellar tissue. These results thus provide some basis for postulating a neurotransmitter function for brain CRF.

Evidence for a functional role of cerebral CRF is provided by reports of changes in the cerebral concen-

trations of CRF in various brain regions following stressful treatments. A preliminary report indicated a doubling of CSF concentrations of irCRF following 15 min footshock in rats²⁹. Chappell et al. studied irCRF in 32 brain regions of the rat following 3 h of cold restraint (acute stress) or a 13-day series of different 'unpredictable' stressors (chronic stress)⁴⁷. The statistically significant changes observed in the content of irCRF were: increases in the LC by acute and chronic stress and in the periventricular nucleus and anterior hypothalamic area by chronic stress; and decreases in the median eminence-arcuate nucleus area by acute and chronic stress, in the medial preoptic area by acute stress and in the dorsal vagal complex by chronic stress. The decreases following acute stress presumably indicate increased release (and hence loss) of CRF from those areas, but the increases are difficult to interpret. After chronic stress, decreases may still reflect enduring release, while increases may reflect increased synthesis to compensate for the increased release. Apparently, prolonged stress is necessary to produce such effects, because Deutch et al. found no statistically significant changes in irCRF concentrations in several dopamine-rich brain areas after 20 min of footshock (0.2 mA circa 1 per s)⁶⁵. Nevertheless, Owens et al. found that a single dose of the anxiolytic drugs, alprazolam (1 mg/kg) or adinazolam (10 mg/kg) increased hypothalamic concentrations of CRF and decreased those in the locus coeruleus 1 h later¹⁷⁸, effects opposite to those observed following acute and chronic stress discussed above.

3.2. CRF receptors

Using CRF and CRF analogs, it has been demonstrated that the characteristics of cerebral CRF-binding sites are similar to those described for the pituitary. Binding of CRF or CRF analogs in both rat and primate brain was saturable, reversible and of a high affinity with a dissociation constant (K_D) in the nanomolar range^{48,62}.

CRF-binding in the pituitary stimulates the activation of adenylate cyclase and the accumulation of cyclic adenosine monophosphate (cAMP), an action thought to regulate the release of ACTH^{89,260}. Similarly, binding of CRF in the brain stimulates activation of adenylate cyclase^{2,48,244,260}. CRF stimulation of adenylate cyclase is regulated by guanine nucleotides and divalent cations, just as occurs with other receptors coupled to adenylate cyclase⁴⁸. The potency of CRF analogs in activating brain adenylate cyclase correlated well with their potency in activating ACTH secretion from pituitary cells and the adenylate cyclase activation by CRF in brain tissue was blocked by the CRF-antagonist, α -helical CRF₉₋₄₁ (ahCRF)⁴⁸. Nevertheless, Chen et al. found that the

correlation between CRF-stimulated adenylate cyclase and binding sites in various brain regions was poor⁴⁸.

The regulation of cerebral CRF-binding sites appears to be different from that in the pituitary. The number of pituitary CRF-binding sites progressively decreases following adrenalectomy, accompanied by a decreased activation of adenylate cyclase^{262,263}. Interestingly, although the number of binding sites and cyclase activation were decreased by adrenalectomy, there was a 3-fold increase in CRF-stimulated ACTH release in vitro²⁶³. Consistent with the effects of adrenalectomy, the number of binding sites was increased by corticosterone¹⁰⁸ or immobilization stress¹⁰⁹. Chronic infusion of CRF itself desensitized the pituitary CRF receptor-adenylate complex; there were decreased numbers of binding sites, decreased activation of adenylate cyclase and decreased ACTH secretion in response to CRF²⁶⁴. However, neither the number of binding sites nor the coupling to adenylate cyclase in the brain was significantly altered by any of these treatments^{108,109,262,263}.

Although adrenalectomy has no effect on the number of CRF-binding sites or CRF-stimulated adenylate cyclase activity in the brain, alterations in the number of brain CRF-binding sites have been observed under certain conditions. In Alzheimer's disease victims, there was a decrease in irCRF in frontal, temporal and occipital cortices^{21,64} that was accompanied by an increase in the number of binding sites⁶⁴. Consistent with the cholinergic deficit in Alzheimer's disease, chronic atropine treatment of rats resulted in an increase in CRF-binding sites in the cerebral cortex⁶⁰. Chronic treatment with the antidepressants, imipramine or desmethylinipramine did not alter CRF binding in many regions of brain, except in the brain stem, where imipramine increased binding⁹⁶. Chronic treatment with diazepam, alprazolam or adinazolam (10 mg/kg daily for 28 days) resulted in decreased CRF-binding in the frontal cortex and hippocampus⁹⁶. Chronic treatment with cocaine (20 mg/kg for 15 days) decreased CRF-binding in medial prefrontal cortex, nucleus accumbens, olfactory tubercle, frontal cortex and amygdala, and increased it in the substantia nigra and ventral tegmental area (VTA)⁹¹. Intracerebral 6-hydroxydopamine treatment (combined with desmethylinipramine to confine damage to dopaminergic cells) increased CRF-binding in most regions containing dopaminergic neurons, including medial prefrontal cortex, caudate, globus pallidus, anterior hypothalamus, medial forebrain bundle, substantia nigra and VTA⁹¹. Thus 6-hydroxydopamine treatment prevented almost all the effects of cocaine⁹¹. These results indicate that the CRF system is a dynamic one, capable of making compensatory changes to perturbations in normal function.

3.
tit
ac
la
ar
C
to
sf
ac
4.
4.
tc
st
C
M
th
A
th
te
el
T
al
za
tr
re
tc
pl
el
ar
re
w
(
sl
tr
n-
β-
b
A
A
p
o
tl
tl
fr
b
a
n

3.3. Neurochemical responses to administered CRF

One recent study examined changes in glucose utilization with the 2-deoxyglucose procedure 10 min after i.c.v. administration of CRF²¹⁰. Changes were observed in a large number of brain areas, including locus coeruleus and the median raphe nucleus. However, the dose of CRF used was so high (more than 30 μg) that it is difficult to interpret these changes in physiological terms. Responses of the various neurotransmitter systems to the administration of CRF will be discussed below.

4. PHYSIOLOGICAL EFFECTS OF ADMINISTERED CRF

4.1. Endocrine effects of administered CRF

CRF is generally considered to be the primary activator of the HPA axis. Although other factors have been shown to possess corticotropin-releasing activity, the CRF of Vale et al.²⁴⁶ appears to be the most potent¹⁸⁷. Moreover, studies with specific CRF antagonists indicate that CRF is the major factor causing elevations of plasma ACTH during stress. Rivier et al. initially demonstrated that immunoneutralization of endogenous CRF by systemic administration of CRF antiserum could prevent the elevation of plasma ACTH in rats exposed to ether¹⁹³. This result was replicated by Ono et al.¹⁷⁷ and Nakane et al.¹⁶⁴ and others have shown effective immunoneutralization following cold-water swims, immobilization or trauma due to bone fracture¹⁶⁴ and formalin or brief restraint¹⁵⁰. Curiously, Ono et al. reported that antisera to CRF administered i.c.v. attenuated elevations of plasma ACTH¹⁷⁷. Although i.c.v. antiserum was less effective than i.v., it is not clear why i.c.v. antisera had any effect at all. Subsequently, Rivier et al. reported reversal of ether-induced elevations of plasma ACTH with peripheral administration of the antagonist, ahCRF (1 mg i.v.)¹⁹⁴. In unstressed rats, Conte-Devolx et al.⁵⁴ showed that antibody to CRF decreased plasma concentrations of ACTH and β -endorphin, but not α -melanocyte-stimulating hormone (α -MSH), suggesting that β -endorphin, but not α -MSH is co-released with ACTH by CRF.

VP has a powerful synergistic action on CRF-induced ACTH secretion^{8,194}. VP may have some intrinsic ACTH-releasing activity^{150,194}, but its ability to elevate plasma ACTH in conscious animals is largely dependent on the presence of CRF^{187,192}. The role is illustrated by the study of Linton et al.¹⁵⁰ mentioned above, who found that immunoneutralization of VP decreased the effects of formalin or restraint on the elevations of plasma ACTH, but to a lesser extent than CRF antiserum. However, VP antiserum complemented the effects of CRF antiserum.

Because the anterior pituitary is outside the blood-

brain barrier, the HPA is best activated by systemic administration of CRF. However, CRF administered i.c.v. also activates the HPA axis in rats (0.3–1.0 μg)^{175,254}, mice (1 μg)⁶⁹ and monkeys (0.8–80 μg)^{116,121,196}. It is not clear whether this occurs because of leakage of the CRF from the cerebral ventricles to the periphery or because of a direct action within the CNS. In one study, a high dose of CRF (10 μg) i.v. elevated plasma corticosterone in dexamethasone-treated rats⁶¹, suggesting that there may be a direct adrenal effect of CRF at this high dose. However, according to Ono et al.¹⁷⁶ there may be a positive ultrashort feedback loop for CRF such that intracerebral CRF stimulates its own release. If this is the case, i.c.v.-administered CRF would stimulate the release of endogenous CRF. This mechanism would explain the HPA activation following i.c.v. CRF. Because i.c.v.-administered CRF might not reach all the relevant sites in the brain, an activation of endogenous CRF systems could account for the potency of i.c.v.-administered CRF. However, negative feedback of CRF on its own release has been suggested by *in vitro* studies⁴⁵, although other studies have been inconclusive¹⁸⁷.

I.c.v. CRF administration (0.5–10 μg) inhibits the secretion of luteinizing hormone (LH)^{175,188,241} and growth hormone (GH)^{127,175,189}, but not follicle-stimulating hormone (FSH)^{188,241}, thyroid-stimulating hormone¹⁷⁵ or prolactin (Prl)^{177,241}. Prolonged i.c.v. CRF administration in rats decreased LH and testosterone secretion in male rats¹⁵⁹. I.v. administration of a single dose of CRF (10–100 μg) had no effects on LH¹⁸⁸ or GH secretion^{127,189} in rats. However, in ovariectomized rhesus monkeys¹⁷⁴ and women¹³, intravenous infusion of CRF inhibited LH and FSH secretion. Moreover, chronic i.v. administration of CRF (5 $\mu\text{g}/\text{day}$) to rats decreased LH, but did not alter testosterone or Prl secretion¹⁹⁰. The chronic effect on LH appeared to be mediated by adrenal steroids, because it was mimicked by ACTH administration and the effect of ACTH was absent in adrenalectomized animals¹⁹⁰.

By contrast with the lack of effect of i.c.v. CRF, i.v. CRF increased Prl secretion in ovariectomized rhesus monkeys (100 μg)²⁵³ and rats (10 μg)¹⁶². The effect in monkeys was blocked by prior administration of naloxone, suggesting a role for endogenous opiates.

The effect of i.c.v. CRF on LH secretion in rats appears to be a central one, because CRF inhibited gonadotropin (GnRH) secretion from hypothalamic slices *in vitro*^{87,170}, and GnRH secretion into the portal blood *in vivo*¹⁸³. Endogenous opiates may also be involved because naloxone⁶ or antiserum to β -endorphin¹⁸² attenuated the effect of i.c.v. CRF on LH secretion in rats. Naloxone also reversed the effect of

peripherally administered CRF on LH and FSH secretion in rhesus monkeys⁹⁰ and women¹³. An involvement of endogenous opioids in GnRH secretion is consistent with the data on the effects of CRF on sexual behavior (see Ref. 218 below). The effect of CRF on GH secretion appears to involve an inhibition of somatostatin (SS) secretion, because peripheral administration of antisera to SS prevented this effect of i.c.v. CRF (10 μ g)^{127,191}. However, CRF has been reported to stimulate SS release from fragments of median eminence *in vitro*¹.

CRF has minor effects on insulin and glucagon secretion. According to one report, plasma concentrations of insulin were slightly reduced and those of glucagon slightly increased 2 min after i.v. CRF, whereas the reverse changes were observed 10 min after CRF¹²⁶. These effects of CRF may be exerted on pancreatic cells, but the results have been conflicting (see Ref. 126). CRF has also been reported to inhibit the secretion of TRH from rat hypothalamus *in vitro*¹⁶⁰.

Each of the above-mentioned changes in hormone secretion is commonly observed in stress^{72,154}. A physiological role for CRF in mediating these stress-induced changes in hormone secretion is suggested because various CRF antagonists can reverse or attenuate the effects of stressors. Electric shock-induced decreases in GH¹⁹¹ and LH secretion in male rats¹⁹⁵ were prevented by the administration of ahCRF (100 μ g i.c.v.). These effects were apparently central because 500 μ g of ahCRF administered i.v. had no significant effects^{177,195}. Ono et al.¹⁷⁷ showed that i.c.v. (but not i.v.) administration of antibody to CRF (3 μ l) prevented the ether-induced decrease in GH secretion, along with the suppression of the elevation of plasma ACTH. In this study, CRF antisera did not alter basal LH secretion or the stress-induced changes. I.c.v. antiserum to CRF did not reverse the stress-related elevations of plasma Prl¹⁷⁷.

4.2. Autonomic effects of administered CRF

I.c.v. CRF increases the plasma concentrations of norepinephrine (NE) and epinephrine (EPI) in both rats (35 μ g)³⁷ and dogs (120 μ g)³⁵. Accompanying these changes in circulating catecholamines were increases in plasma glucose and glucagon and bodily oxygen consumption^{37,38} and mean arterial pressure (MAP) and heart rate⁸⁴. Grosskreutz and Brody⁹⁷ found i.c.v. CRF (0.15–3.4 μ g) to increase heart rate and vascular resistance in non-muscular vascular beds, both of which could be caused by sympathetic activation. In dogs, plasma VP was also elevated by 120 μ g CRF i.c.v.³⁵. An adrenomedullary involvement in these changes is indicated because i.c.v. CRF (0.4–40 μ g) also increased the electrophysiological activity of the splanchnic (adrenal) nerve¹³⁷. However, the sympathetic nervous system is

probably activated also, because the autonomic ganglionic blocker, chlorisondamine, prevented the CRF-induced (10 μ g i.c.v.) increases in plasma glucose, NE and EPI³⁸ and attenuated or reversed those in MAP and heart rate^{84,85}. The effects of CRF are almost certainly centrally mediated, because hypophysectomy or adrenalectomy did not alter the elevations of plasma glucose³⁸, nor did these treatments or dexamethasone pretreatment alter the effects on MAP and heart rate⁸⁵. Moreover, i.v. CRF (35 μ g) decreased MAP, apparently with a reflex tachycardia⁸⁵ and peripheral administration of antibody to CRF which blocked the elevation of plasma ACTH by i.c.v. CRF failed to prevent the elevations of NE and EPI³⁶.

To investigate the intracerebral sites of action of CRF, Brown³⁴ injected CRF (1 μ g) into 50 different brain sites. Although some sites were unresponsive to CRF, none of the sites showed elevations of plasma NE greater than those observed from third ventricle injections.

A physiological role for CRF in activating the sympathetic nervous system is suggested by studies with CRF-antagonists. I.c.v. ahCRF (100 μ g) did not alter basal concentrations of NE or EPI⁴⁰, but reversed the i.c.v. CRF-induced elevations of plasma catecholamines³⁹. The same dose of ahCRF reversed the ether-induced increase in plasma EPI, but not that of NE³⁹. This could be interpreted to mean that the activation of the sympathetic nervous system is more sensitive than the adrenal medulla to the blockade of central CRF-receptors.

Interestingly, Fisher has recently provided evidence that i.c.v. CRF (1 or 10 μ g) modifies baroreflex control of heart rate and that this effect is prevented by blockade of the vagus (with atropinemethylnitrate), but not of sympathetic output (with propranolol)⁸³. Thus i.c.v. CRF may affect both the sympathetic and the parasympathetic nervous systems.

By contrast with the increased blood pressure caused by i.c.v. CRF, systemic CRF (10–35 μ g) causes hypotension^{85,130} and, according to one study, bradycardia¹³⁰. The bradycardia, but not the hypotension, was prevented by hypophysectomy, dexamethasone or naloxone, but not by vagotomy. Thus Kiang and Wei¹³⁰ speculated that the bradycardia was primarily due to secretion of opioid peptides from the pituitary, whereas the hypotension was largely due to dilation of the mesenteric circulation, increasing blood flow to the gut.

CRF has also been implicated in the pyrogenic activity of interleukin-1 (IL-1). IL-1 injected either peripherally or i.c.v. elevates the body temperature of rats. This effect is apparently due to a sympathetic activation of metabolism in brown adipose tissue⁵⁸. Intriguingly, i.c.v. ahCRF (25 μ g) reversed the effect of human recombinant

IL-1 β (50 ng i.c.v.) on body temperature and resting oxygen consumption¹⁹⁹. This suggests that the thermogenic effects of IL-1 β are exerted by release of brain CRF, which in turn activates the sympathetic nervous system to increase brown adipose tissue metabolism. Surprisingly, the pyrogenic effect of IL-1 α was not reversed by ahCRF and, unlike that of IL-1 β , appeared to be sensitive to prostaglandin synthesis inhibitors⁴³.

4.3. Electrophysiological effects of administered CRF

Eberly et al.⁷⁶ reported regionally specific responses to iontophoretically applied CRF. Inhibition of cell firing was recorded in the thalamus and lateral septum, whereas excitation occurred in the cortex and hypothalamus. I.c.v. CRF also affected the electrographic activity of rats^{77,153,259}. In the study by Ehler et al.⁷⁷ low doses (0.01–0.1 μ g) produced behavioral arousal accompanied by activation of the EEG characteristic of arousal. Higher doses (1–25 μ g) also produced electrographic symptoms of arousal, but after a delay of 1–3 h seizure activity occurred. Marrosu et al.¹⁵³ compared the activity of rat and ovine CRF. Both activated the EEG at doses of 0.1 or 1.0 μ g and caused spiking at 10 μ g, but the latter effect was confined to the hippocampus with rat but not with oCRF. None of these effects of CRF were altered by naloxone. I.c.v. administration of CRF (0.01–0.1 μ g) produced decreases in slow-wave sleep⁷⁸.

The effects of i.c.v. CRF on the spontaneous and sensory-evoked activity of LC neurons has been examined in rats. In anesthetized animals, i.c.v. CRF increased the spontaneous discharge rate of LC neurons. This effect was statistically significant at doses of 1 and 3 μ g, but not 0.3 μ g^{248,250}. Similar, but less consistent effects were observed with direct administration of CRF onto the LC. I.c.v. CRF decreased the excitatory component and increased the inhibitory component of the sensory-elicited response of LC neurons²⁴⁸. The overall effect of CRF on the sensory-elicited response of these cells was to disrupt the pattern of response and to decrease the signal-to-noise ratio. In unanesthetized rats, i.c.v. CRF also increased the spontaneous discharge rate²⁴⁹, at doses of 1 or 3 μ g, but not 0.3 μ g. Although CRF did not significantly affect either the excitatory or inhibitory responses to sensory stimuli, the overall effect was to decrease the signal-to-noise ratio. These electrophysiological data complement the neurochemical findings of increased production of NE catabolites following intracerebral CRF administration (see below). Because the LC has been postulated to regulate arousal or vigilance states, the ability of CRF to increase the spontaneous activity of LC neurons suggests that CRF might act to increase arousal or vigilance²⁵⁰. The behavioral significance of the ability of CRF to disrupt

neuronal responses in the LC to sensory stimuli is presently unclear.

That endogenous CRF might act to regulate the increase in LC discharge rate observed during stress is suggested by the ability of ahCRF (50 μ g) to block the nitroprusside-induced increase in LC firing^{247,251}.

In hippocampal slices, high concentrations of CRF (more than 0.25 μ M) usually depolarized both CA1 and CA3 pyramidal neurons, accompanied by increases in the spontaneous firing rate^{5,213}. At lower concentrations (0.01–0.2 μ M) CRF also reduced the magnitude and duration of afterhyperpolarizations after spontaneous or current-induced outbursts of action potentials. The enhanced discharge activity of hippocampal neurons is consistent with the CRF-induced changes in electrographic activity recorded in vivo described above.

4.4. Gastrointestinal effects of administered CRF

CRF administration has been shown to have widespread effects on gastrointestinal function. The effects of CRF include a whole spectrum of activities known to be affected in stress, including gastric acid secretion, bowel emptying and a variety of measures of gastrointestinal motility. The effects are complex and appear to depend upon the species and to some extent on the form of the stress. Generally, stress or CRF inhibit gastric acid secretion and gastric emptying, while stimulating large bowel transit and fecal excretion.

4.4.1. Gastric acid secretion. Taché et al.²³⁶ first showed that intracisternal (IC) CRF (1.5, 5 or 15 μ g) inhibited gastric acid secretion in rats. A direct central effect of CRF was suggested because its effect was present in hypophysectomized animals and local application of CRF (1.5 μ g) into the lateral hypothalamus, but not the dorsomedial frontal cortex was effective. The effect of IC CRF was prevented by vagotomy, yohimbine treatment or adrenalectomy. In subsequent work, the effect of CRF (1–4 μ g) was localized to the PVN or ventromedial hypothalamus¹⁰³. Similar effects were found with lateral ventricle injection of CRF (0.6 or 12 μ g)⁶⁶. In these studies, the effects of i.c.v. CRF were reversed by chlorisondamine, bretylium, adrenalectomy or i.c.v. ahCRF (50 or 100 μ g), but not by vagotomy or naloxone⁶⁶.

Lenz et al.^{141,142} observed a similar decrease of gastric acid secretion with third ventricle application of CRF (0.6–36 μ g/kg) in dogs. As in rats, the effect was reversed by chlorisondamine, but only partially by naloxone or a VP antagonist and not affected by vagotomy. These effects of i.c.v. CRF can best be explained by activation of the sympathetic nervous system which occurs in parallel with these effects at these doses of CRF¹⁴².

I.v. CRF also inhibited gastric acid secretion in rats

(25–150 $\mu\text{g/kg}$)^{144,237} and anesthetized and unanesthetized dogs (25–100 and 6–60 $\mu\text{g/kg}$, respectively)¹³¹. This effect in rats was not altered by naloxone (5 mg/kg) or indomethacin (10 mg/kg) pretreatment, nor by adrenalectomy or hypophysectomy, but was partially prevented by vagotomy²³⁷.

A role for endogenous CRF in the effects of stress on gastric acid secretion is suggested, because two groups have observed reversal of stress-related effects by ahCRF. Stephens et al.²²⁷ have shown that IC ahCRF (10 or 50 μg) administered to rats reversed the surgery-related decrease and Lenz et al.¹⁴⁴ found ahCRF (5–50 μg) i.c.v., but not i.v., reversed the partial restraint-induced inhibition of gastric acid secretion.

4.4.2. Gastric emptying. In rats, i.c.v. (0.6–10 μg)¹⁴³,²⁵⁸ or IC CRF (0.3–1 μg)²³⁸ decreased gastric emptying of saline. Taché's group found that the effects of IC CRF were not prevented by naloxone treatment, nor by adrenalectomy, but were reversed by vagotomy. Moreover, i.v. administration of antiserum to CRF did not prevent the effect of IC CRF administration²³⁸. However, Lenz et al.¹⁴³ found that the effects of lateral ventricle infusion of CRF (0.6 or 6 μg) were completely abolished by chlorisondamine, naloxone or bretylium treatment, but not by adrenalectomy, hypophysectomy or truncal vagotomy, suggesting the involvement of the sympathetic nervous system. Surgical stress or partial restraint stress also inhibited gastric emptying and these effects were reversed by i.c.v. ahCRF (60 μg)^{144,239}.

I.v. CRF (0.15–10 μg)^{144,238,258} or i.p. (6 μg)¹⁴³ also inhibited gastric emptying in rats. This effect of CRF was prevented by i.v. antiserum to CRF²³⁸, but not altered by bretylium or chlorisondamine¹⁴³.

However, in the mouse, gastric emptying of a non-nutritive meal was stimulated by i.c.v. CRF (0.15 μg)^{41,100}, but not by the same dose of CRF administered IP nor by corticosterone (300 ng) or ACTH (375 μU)⁴¹. The effects of i.c.v. CRF resembled those of exposure to 20 min acoustic or cold stress^{41,100}. The effects of acoustic stress were reversed by i.c.v. ahCRF (200 ng) or antiserum to CRF⁹⁸. Curiously, the effects of acoustic or cold stress or i.c.v. CRF were reversed by antiserum to CRF-administered IP⁴¹.

In the dog, gastric emptying of a non-nutritive meal was inhibited by i.v. CRF (0.6–6 $\mu\text{g/kg/h}$)^{131,179,180}. I.c.v. CRF (0.7–3.0 $\mu\text{g/kg}$) had no such effect¹⁸⁰. The effects of i.v. CRF were not altered by naloxone¹⁷⁹ or propranolol¹⁸⁰.

4.4.3. Gastrointestinal motility. I.c.v. CRF (0.3–6.0 μg) inhibited small bowel transit and markedly increased large bowel transit in the rat^{143,144,260}. These effects were reversed by chlorisondamine or vagotomy, but not by bretylium¹⁴³. Whereas the effect on the small bowel was

reversed by naloxone, the large bowel effect was not¹⁴³. Partial body restraint mimicked these effects of i.c.v. CRF^{144,258}. The effects of both CRF and partial restraint were reversed or attenuated by i.c.v. ahCRF (50 μg)¹⁴⁴. The antagonist was ineffective against restraint or i.c.v. CRF when given i.v.¹⁴⁴. In the study of Williams et al.²⁶⁰ CRF (0.3 μg) or partial restraint stress also increased fecal excretion. The effect of the restraint was reversed by ahCRF (50 μg i.c.v.). Moreover, i.v. or i.c.v. ahCRF (50 μg) reversed the effects of partial restraint on small and large bowel transit and on fecal excretion.

I.v. (1–10 μg)²⁶⁰ or IP CRF (6 μg)¹⁴⁴ stimulated large bowel transit, but inhibited²⁶⁰ or did not alter small bowel transit¹⁴⁴. These effects were not altered by bretylium or chlorisondamine¹⁴⁴.

Migrating myoelectric complexes (MMCs) can be recorded by cutaneous electrodes on the abdomen. They are the basic motor pattern of stomach and small intestine, characterizing the fasted state. The activity is cyclic (90–120 min) and is involved in mixing the contents of the bowel and in movement of matter and nutrient absorption in the upper gut. Their occurrence in fasted dogs was inhibited by 60 min acoustic stress⁹⁹. I.c.v. CRF (0.02–0.1 $\mu\text{g/kg}$) suppressed gastric MMCs for 4–5 h^{42,99}. I.v. CRF (0.1 $\mu\text{g/kg}$) had no such effect⁴². The effect of i.c.v. CRF was abolished by thoracic vagotomy⁹⁹.

In fed sheep, CRF decreased antral activity²⁰⁰. IC CRF (0.1–1 μg) decreased 2-deoxyglucose- or TRH-stimulated gastric contractility (the antral motor response) in the rat⁸⁸. I.v. CRF was also effective, but approximately 10 times less potent.

4.4.4. Gastric ulceration. Intracisternal administration of CRF (5–10 μg) did not elicit gastric lesions^{95,135,165}. However, intrahypothalamic injection of CRF (2 μg per side) or 5 μg i.c.v. prevented the lesions caused by 1–4 h cold restraint^{102,135}. An i.c.v. injection of 50 μg ahCRF reversed the effect of CRF, but not that of cold restraint¹³⁵. These results suggest that CRF has an amelioratory effect on stress-induced gastric lesions.

Apparently, CRF can have both central and peripheral effects on the gastrointestinal function. Judged by the use of CRF antagonists, these effects are independent. CRF decreases gastric emptying, gastric acid secretion and small bowel transit, while increasing large bowel transit and fecal excretion. An exception is the mouse, in which gastric emptying of a non-nutritive meal was increased. However, in most cases the effects of CRF resemble those of various stressors. The effects of i.c.v. or IC CRF seem to be partly mediated by the sympathetic nervous system, with the possible participation of the vagus. On the other hand the effects of i.v. CRF seem to be independent of the sympathetic nervous system, but may depend upon an intact vagus.

5
t
a
t
u
f
e
ic
s
b
is
b
s
o
r
a
p
5.
o
r
d
at
i.
ar
di
C
C
be
ef
w.
lo
ar
ar
in
0.
in
sp
cr
ca
hc
of
(0
in
fie

5. BEHAVIORAL RESPONSES TO ADMINISTERED CRF

CRF administered to animals elicits a number of behavioral responses. In almost every case intracerebral administration appears to be necessary to elicit significant behavioral effects. Peripheral administration is either ineffective or much greater doses are necessary to produce effects, which may even differ from those elicited by intracerebral injections. Many of the behavioral responses to CRF resemble those observed during stress. Consistent with this, the effects of CRF are frequently opposite to those observed following administration of benzodiazepines and the latter have often been found to reverse the effects of CRF. Moreover, in some cases, CRF-antagonists have been found to reverse or attenuate stress-induced changes. In this section, we review the behavioral data reported following CRF administration and other relevant observations on those particular behaviors.

5.1. Locomotor activity

The effect of CRF on locomotor activity is dependent on both the dose of CRF and the testing conditions. In rats tested in a familiar environment, i.c.v. CRF produced a dose-dependent activation of locomotor activity at doses between 0.1 and 10 μg ^{27,44,211,230}. This effect of i.c.v. CRF was apparently independent of pituitary-adrenal activation because it was not blocked by either dexamethasone at a dose that prevents the increase in plasma corticosterone induced by CRF^{27,32}, nor by hypophysectomy⁷⁵. Further, i.v. CRF (8 μg) did not alter locomotor activity when tested under similar conditions²⁷. The CRF antagonist, ahCRF, blocked the CRF-induced increase in locomotion, suggesting that CRF affects this behavior through CRF receptors³². The benzodiazepine antagonist, Ro 15-1788, did not alter the effect of i.c.v. CRF on behavior in this paradigm³³. CRF was 10 times more potent when injected directly into the locus coeruleus as compared to the cerebral aqueduct⁴⁴.

In an open field test, the behavioral responses of an animal in a relatively large and open novel environment are observed. In this test, low doses of i.c.v. CRF increased locomotor activity of rats (0.01 μg , but not 0.001 or 0.0001 μg)²³⁰ or mice (0.2 μg)¹⁴⁰. The increase in activity was primarily in the central region in both species. However, higher doses of CRF (1–2 μg) decreased activity^{25,26,114,140,230}. When observed in a novel cage with wood shavings present, CRF (3 μg) increased horizontal locomotor activity but decreased the number of rears²¹¹. In mice, CRF injected into the hippocampus (0.01 μg each side) or amygdala (0.02 μg each side) increased locomotor activity in the center of an open field¹³⁹. However, Rosenthal and Morley¹⁹⁷ found no

effect of i.c.v. CRF (0.01–10 μg) in mice deprived of food during 18 h.

The increased activity observed at lower doses of CRF in an open field resembles that observed following exposure of animals to a stressor^{139,140,198}. Further, the locomotor-activating effect of CRF in mice was reversed by pretreatment with 2 mg/kg diazepam, a dose that had no sedative effects when administered in the absence of CRF¹⁴⁰. Thus, low doses of CRF have stress-like properties increasing locomotor activity in the open field.

5.2. Ingestive behavior

I.c.v. CRF inhibited feeding in food-deprived animals in both familiar and novel environments. At doses greater than 1 μg , i.c.v. CRF decreased food intake in food-deprived rats^{9,94,134,163} and in mice¹⁹⁷. The CRF-induced decrease in feeding was not altered in hypophysectomized animals (5 μg CRF)¹⁶³, nor by pretreatment with dexamethasone (0.5 μg CRF)²⁷. CRF (5 or 10 μg , but not 1 μg) also inhibited muscimol-, norepinephrine- and dynorphin-induced¹⁴⁵ and ethylketocyclazocine-induced feeding (5 μg)⁹⁴. The inhibitory effect of i.c.v. CRF on feeding is similar to that observed following restraint for 1 h¹³⁴. Moreover, ahCRF (50 μg) -injected i.c.v. attenuated this effect of restraint¹³⁴, suggesting that endogenous CRF is involved in the restraint-induced decrease in feeding. Interestingly, interleukin-1 (1–25 $\mu\text{g}/\text{rat}$ IP) induces anorexia in rats. CRF appears to be implicated in this effect, because i.c.v. antiserum to CRF (10 μl) prevented the effects of IL-1 β (2 μg IP)²⁴⁵.

In an open field in which a pellet of food was secured to the center (and presumably more aversive) region, CRF (0.5 and 1.0 μg , but not 0.1 μg) decreased both the number of approaches to the food and the average amount of food consumed per approach^{25,26}. This effect was opposite to that observed following administration of benzodiazepines²⁴ suggesting that CRF enhances the anxiogenic nature of the novel environment. However, because similar doses of CRF inhibit feeding in a familiar environment, its ability to decrease feeding in an open field may be independent of effects on the animal's reaction to the novel environment.

Interestingly, CRF at a lower dose (0.1 μg) increased feeding in 24-h food-deprived animals⁹⁴. The ability of CRF to exert opposite effects on the same behavior depending on dose resembles that observed with locomotor activity in the open field.

5.3. Antinociception

Human patients injected with CRF i.v. (1 $\mu\text{g}/\text{kg}$) reported less postoperative pain (molar extraction) than those that received placebo¹⁰⁴. Likewise, rats injected i.v. with oCRF (150 μg) showed almost as much antinoci-

sponsiveness as those that received 2.5 mg/kg morphine in the hot-plate test¹⁰⁴. This effect is probably due to release of β -endorphin, because plasma concentrations were elevated by the oCRF infusion¹⁰⁴ and the antinociceptive effects were reversed by hypophysectomy or dexamethasone treatment¹⁰⁶. Doses of CRF up to 3 μ g i.c.v. failed to alter latencies of rats to respond in either the hot-plate or the tail-flick tests for analgesia²¹¹.

CRF (120 μ g/kg) administered SC decreased the hyperalgesia, edema and hyperthermia observed in the carrageenan model of inflammation¹⁰⁵. These effects were blocked by adrenalectomy but not by hypophysectomy. CRF also had an antinociceptive effect when injected directly into the inflamed hindpaw (0.4 μ g), but the local injections had no effect on measures of inflammation.

5.4. Grooming

I.c.v. CRF increased grooming in rats at doses above 0.3 μ g (0.3–20 μ g) when tested in either the open field or familiar testing chambers^{25,26,27,74,114,163,207,211,212,254}. Doses below 0.3 μ g were ineffective in most of these studies^{25,74,163,254}, although 0.1 μ g was effective when rats were tested in a shock chamber²¹² or in the social interaction test⁷⁰. Thus, the threshold for CRF to induce grooming may depend on the testing environment.

The CRF-induced increase in grooming is not dependent on the pituitary-adrenal axis because this effect was not inhibited by hypophysectomy¹⁶³ or dexamethasone pretreatment^{27,74}. Nor did SC administration of CRF increase grooming behavior in rats^{27,211}. CRF-induced grooming was prevented by pretreatment with naloxone⁷⁴. Although CRF increases grooming in rats, very little response was observed in mice injected with up to 1 μ g i.c.v.⁷⁴. In fact, one report found decreases in mice that had been briefly anesthetized before testing with 0.01–10 μ g CRF i.c.v.¹⁹⁶. It is unclear whether this represents a difference between the species in the ability to respond to the peptide or in the sensitivity of the mice to CRF.

Effects on grooming may be related to stress, because rats exposed to a novel environment display increased grooming behavior that habituates with repeated exposure to the same environment⁴⁹ and is attenuated by benzodiazepine pretreatment⁷³.

5.5. Conflict tests and other tests of anxiety

The effect of CRF has been examined in a number of tests commonly used in the study of anxiety and anti-anxiety drugs. These tests include the Geller–Seifter conflict test, the social interaction test and the acoustic startle response. As described above, CRF decreased the number of approaches and the amount of food eaten in

an open field, an effect opposite to that observed following benzodiazepine administration^{25,26}.

5.5.1. Geller–Seifter conflict test. In the Geller–Seifter conflict test, anxiolytic agents increase behavioral responding (lever press for food) in the presence of footshock (i.e. punished responding), without effects on responding in the absence of the aversive stimulus (unpunished responding). In this test, i.c.v. CRF (0.5 or 1.0 μ g) decreased both punished and unpunished responding^{30,33}. Dexamethasone pretreatment did not inhibit the effect of CRF in this test³². AhCRF blocked this effect of CRF, suggesting that CRF acts through specific receptors³¹. Chlordiazepoxide (CDP; 5 mg/kg) completely antagonized the effect of CRF on the conflict component and partially reversed the CRF-induced decrease in the unpunished component^{30,33}. At this dose, CDP had no effect on unpunished responding in the absence of CRF, but did increase punished responding. The benzodiazepine antagonist, Ro 15–1788, also antagonized the inhibitory effect of CRF on punished responding at a dose that did not affect punished responding in the absence of CRF³³. This may reflect some partial agonist properties of Ro 15–1788. By contrast, a benzodiazepine inverse agonist, FG 7142, decreased punished responding and potentiated the effects of CRF in this paradigm³³.

These results are consistent with CRF having a stress-like or anxiogenic effect. The simple explanation that CRF enhances the sensitivity of the animal to the pain of the electric shock used in this test is unlikely because CRF did not increase the animal's sensitivity to noxious stimuli in the hot plate and tail flick tests^{30,211,212}. In fact, as mentioned above i.v. CRF caused apparent analgesia in rats in the hot plate test¹⁰⁴. However, the fact that unpunished responding was also depressed suggests that CRF may have produced a generalized inhibition of responding. The effects of CRF could also be related to its inhibitory effect on feeding, observed at similar doses (see above). However, the reversal of the effect of CRF on punished responding by doses of CDP or Ro 15–1788 that independently had no effect on this behavior, suggests that CRF may act on anxiogenic mechanisms.

In pigeons i.c.v. CRF (3.0–30 μ g) decreased responding in a shock-motivated operant task¹⁴. This effect of CRF was antagonized by 10 or 30 μ g/kg ahCRF i.c.v.

5.5.2. Social interaction. The social interaction test measures the amount of time that two animals spend in active contact with each other in an open field. Benzodiazepines increase, whereas benzodiazepine-inverse agonists decrease social interaction without affecting locomotor activity⁸¹. I.c.v. CRF (0.1 and 0.3 μ g) decreased the time spent in social interaction in a familiar environment without affecting locomotor activity. This

effect of CRF was reversed by pretreatment with 5 mg/kg CDP⁷⁰.

5.5.3. Acoustic startle. The acoustic startle test measures skeletal musculature contraction in response to a sudden and intense acoustic stimulus. This response is increased under conditions associated with stress or anxiety⁵⁹. I.c.v. CRF at 1 μ g, but not 0.1 or 10 μ g, significantly increased the mean startle amplitude²³³. This effect of CRF was antagonized dose-dependently by CDP (2.5–10 mg/kg)²³³ and by ahCRF (1–25 μ g i.c.v.)²³⁴. The actions of CDP and ahCRF² were not related to non-specific depressant effects, because they did not inhibit the amphetamine- (CDP)²³³ or strychnine-induced (CDP or ahCRF)^{233,234} enhancements of the acoustic startle response. I.c.v. ahCRF (5 or 25 μ g) dose-dependently reversed the effects of conditioned fear on acoustic startle²³⁴. Amygdaloid lesions prevented this effect of CRF¹⁴⁸.

5.5.4. Elevated plus maze. The elevated plus maze is another standard test to assess the anxiolytic/anxiogenic properties of drugs. In this task, File et al. found i.c.v. CRF (0.1 μ g) decreased the time spent on the open arms, suggesting an anxiogenic effect. This effect that was not altered by Ro 15-1788 pretreatment⁸².

5.5.5. Defensive withdrawal. Takahashi et al.²⁴⁰ have used an open field modified by including a metal cylinder into which a rat can retreat. Behavioral testing starts with the rat inside the cylinder and measures of anxiety include the time taken to emerge from the cylinder, the total number of emergences and the mean time spent in the cylinder. AhCRF (50 μ g i.c.v.) significantly decreased the time taken for a rat to emerge from the cylinder for the first time and also decreased the proportion of time the rat spent in the cylinder. When the rats had been familiarized with the apparatus on the previous day, i.c.v. CRF (0.3 μ g) had an anxiogenic effect, increasing the latency to emerge from the cylinder and increasing the proportion of time spent in the cylinder. Peripheral administration of CRF (0.3 μ g IP) was ineffective. The anxiogenic effects of intracerebral CRF in this task have been replicated by Butler et al.⁴⁴ (0.1 μ g into the LC or 1 μ g into the aqueduct) and Yang and Dunn (0.02–0.1 μ g i.c.v.)²⁶⁶

5.5.6. Porsolt swim test. I.c.v. CRF (0.5 μ g) decreased floating time in the Porsolt swim test, indicative of behavioral activation and perhaps anxiety⁴⁴. The CRF was much more effective when injected directly into the locus coeruleus (0.01 μ g).

5.6. Conditioned emotional responses

In a conditioned suppression paradigm, CRF (0.5 μ g, i.c.v.) decreased responding to the conditioned stimulus (CS), suggesting that CRF increased the anxiogenic

character of the test⁵⁰. Although i.c.v. CRF decreased responding in the pre-CS component, the effect was greater in the CS component than in the pre-CS component. Evidence for a role of endogenous CRF in this behavior was obtained from studies with ahCRF, which at i.c.v. doses of 1, 5 and 25 μ g significantly attenuated the CER⁵². These results are consistent with the hypothesis that CRF enhances anxiety.

5.7. Exploratory behavior

The effect of CRF on exploratory behavior in a complex novel environment has also been examined in mice and rats. The testing chamber used was a multi-compartment chamber (MCC) consisting of 9 interconnecting compartments within each of which a wire-mesh sphere was recessed in a hole in the floor, slightly below it^{10,16}. Exposure of rats to restraint or repeated tail-pinch immediately prior to testing or white noise stress during testing decreased the time spent investigating the wire stimuli without affecting measures of locomotor activity¹¹. Very similar results were obtained with prior restraint in mice¹⁶. This effect was reversed by a dose of naloxone that independently had no effect on this behavior^{11,16}. I.c.v. CRF administered to mice (0.005–0.150 μ g)^{16,19} or rats (0.02–0.05 μ g)²²⁶ produced a stress-like response; the investigatory behavior was decreased in the absence of effects on locomotor behavior. The CRF-induced decrease in stimulus interaction time was also blocked by naloxone pretreatment at a dose (0.7 mg/kg) that had no effect in the absence of CRF¹⁶. The effect of CRF on exploratory behavior was also observed in hypophysectomized mice²⁰. I.c.v. injection of ahCRF (10–50 μ g), reversed the restraint-induced decrease in stimulus interaction¹⁷. These results suggest that restraint decreases exploratory behavior by a mechanism that involves the release of endogenous CRF. It is noteworthy that CRF administration also decreased exploratory behavior in primates (see below Ref. 120).

5.8. Shock-induced behaviors

5.8.1. Shock-induced freezing. Rats exposed to a brief footshock display an increase in freezing behavior characterized by a complete lack of movement and a crouched posture²³. I.c.v. CRF (0.3 μ g) had a biphasic effect on this response²¹². It increased the amount of freezing immediately following the shock, whereas at later times (16–20 min), CRF facilitated recovery. Because CRF increased grooming at these later times, it was postulated that the enhanced recovery from the shock-induced freezing was related to the increased grooming activity. Interestingly, CRF did not affect freezing behavior observed following a 60 s exposure to a hot-plate. However, there were a number of differences in the

TABLE I

Responses to intracerebrally administered CRF

Summary of responses to intracerebral administration of CRF. Injections were i.c.v. or intracisternal, except where otherwise indicated. * Injections into the amygdala, hippocampus, or hypothalamus. + Indicates an increase in the parameter, - a decrease (an anxiogenic effect for the Geller-Seifter test and the Porsolt swim test).

Measure	Species	Effective dose of CRF (μ g)	Effect of CRF	Effect of stress	Refs.
Endocrine					
Plasma ACTH	rat	0.5-10	+	+	175
	monkey	180 μ g/kg	+	+	121
Plasma corticosteroids	rat	0.1-1.0	+	+	44, 254
	mouse	1.0	+	+	69
	monkey	0.1-60 μ g/kg	+	+	116, 121, 196
Plasma GH	rat	0.5-10	-	-	127, 175, 189
Plasma LH	rat	1-10	-	-	175, 188, 241
Plasma VP	dog	120	+	+	35
Plasma glucagon	rat	35	+	+	38
Physiological					
Plasma NE, EPI	rat	1-35	+	+	34, 37
	dog	120	+	+	35
	monkey	60 μ g/kg	+	+	121
Plasma glucose	rat	35	+	+	37, 38
Arterial pressure	rat	10	+	+	84, 85
Heart rate	rat	10	+	+	84, 85
EEG	rat	0.01-1.0	+	+	77, 153
		1-25	seizures	+	77, 153
	rat	1-3	+	+	248, 250
Locus coeruleus firing	rat	1-3	+	+	248, 250
Gastrointestinal function:					
Gastric acid secretion	rat	0.6-15	-	-	66, 103, 236
	dog	0.6-36	-	-	141, 142
Gastric emptying	rat	0.6-10	-	-	143, 144, 238, 260
	mouse	0.2-1.0	+	+	41, 100
Gastric ulceration	rat	4-10	-	+	95, 135, 165
Small bowel transit	rat	0.3-6	-	-	143, 144, 260
Large bowel transit	rat	0.3-6	+	+	143, 144, 260
MMCs	dog	0.02-0.1 μ g/kg	-	-	42, 99
Fecal excretion	rat	0.3	+	+	260
Neurochemical					
DA release	rat	1-10	+	+	156
		20	+	+	125
	mouse	0.2-1.0	+	+	69
	pigeon	5.6-30	+	+	14
NE release	rat	1-10	+	+	44, 156
	mouse	0.2-1.0	+	+	69
	pigeon	5.6-30	+	+	14
5-HT release	pigeon	5.6-30	+	+	14
Behavioral					
Locomotor activity:					
Familiar environment	rat	0.1-10	+	+	27, 32, 44, 211, 230, 254
Novel environment	rat	0.2	+	+	198, 230
		>1.0	-	-	25, 26, 114, 211, 230, 254
	mouse	0.01-0.2*	+	+	139, 140
		2	-	-	140
Ingestion:					
Familiar environment	rat	0.1	+	+	94
		0.5-10	-	-	9, 94, 134, 145, 163
	mouse	0.05-10	-	-	197
Novel environment	rat	0.5-1.0	-	-	25, 26
Grooming	rat	0.1-20	+	+	25-27, 70, 74, 114, 163, 207, 211, 212, 254
	mouse	0.1-1.0	0	0	74
Geller-Seifter conflict test	rat	0.5-1.0	-	-	30, 33
Operant responding	pigeon	3-30	-	-	14
Social interaction	rat	0.1-0.3	-	-	70, 81
Acoustic startle	rat	0.5-1 (not 10)	+	+	59, 148, 233, 234
Elevated plus maze	rat	0.1	-	-	82
Defensive withdrawal	rat	0.02-0.3	+	+	44, 240, 265, 266
Porsolt swim test	rat	0.5	-	-	44
Conditioned emotional response	rat	0.5	+	+	50
Exploration - MCC	rat	0.02-0.1	-	-	11, 226
	mouse	0.005-0.15	-	-	16, 17, 19, 20
Shock-induced freezing	rat	0.3	+/-	-	23, 212
			(biphasic)	-	
Shock-induced fighting	rat	0.01-0.5	+	+	242
Sexual behavior					
male	rat	2-4	-	-	152, 220
female	rat	0.5-2	-	-	152, 218, 219, 223
Passive avoidance	rat	0.00003-0.1	-	-	203, 254
		0.1*	+	+	138, 147

testing procedures between the footshock and hot-plate experiments, so that the significance of the different results obtained from these two paradigms is unclear. The shock-induced increase in freezing was partially antagonized by 25, but not 50 μg of ahCRF i.c.v.¹²². This protective effect of ahCRF was also observed when 20 μg was injected before re-exposure to the apparatus in which the rats had been shocked 24 h earlier¹²⁴.

5.8.2. Shock-induced fighting. Exposure of a pair of rats to intermittent footshock elicits boxing (upright posture without physical contact) and fighting behaviors. The frequency of each behavior is dependent upon various parameters including shock intensity. I.c.v. CRF (0.01 and 0.1 μg) increased boxing behavior at lower shock intensities (0.3 and 0.4 mA) and increased fighting behavior at 0.5 mA²⁴². One μg CRF completely disrupted the behavioral response to 0.5 mA. The shock-induced fighting (at 0.6 mA) was reversed by 5 and 25 μg of ahCRF, injected i.c.v., suggesting the involvement of endogenous CRF in regulating this shock-induced fighting²⁴². The minimum doses of CRF and ahCRF that affected behavior in this paradigm were similar to those affecting exploratory behavior in the MCC.

5.9. Sexual behavior

Normal reproductive function is inhibited in stress¹⁵². CRF (0.5–2 μg) inhibited lordosis behavior in ovariectomized, estrogen- or estrogen/progesterone-primed female rats when injected into the mesencephalic gray²¹⁸, arcuate-ventromedial area of the hypothalamus²²³ and the medial preoptic area²¹⁹. Injections of CRF into tissue sites outside these areas had no effect on lordosis behavior, indicating a site-specific effect of CRF on this behavior. In male rats, injections of CRF (2, 4 or 10 μg) into the third ventricle also impaired sexual behavior, with increases observed in the time to mount, the latency to ejaculate, the number of mounts without intromission and the number of intromissions before ejaculation²²⁰. In a sexually motivated learning task, Lee and Sung found biphasic effects of CRF injected into the amygdala¹³⁸. Low doses (0.01 μg) enhanced retention, whereas higher doses (0.1 μg) inhibited it.

5.10. Conditioned avoidance responding

CRF had multiple effects in passive and active avoidance tests depending on the dose and route of administration²⁵⁴. In hypophysectomized rats, which display poor avoidance acquisition, 0.2 or 0.6 μg CRF given SC for 7 days increased acquisition. When tested in the pole jump shock avoidance test, 0.3 and 1.0 μg CRF SC facilitated extinction of the avoidance response in intact and adrenalectomized animals. Because the effect of ACTH or β -endorphin on extinction of the pole jump

response is opposite to that of CRF⁶⁷, the involvement of the pituitary-adrenal axis in the effect of CRF is unlikely. CRF administered peripherally on postpartum days 1–5 improved acquisition of an active avoidance response at 35–37 days of age¹¹².

In the passive (inhibitory) avoidance test, SC administration of 0.03 μg CRF 1 h prior to testing improved performance 24 or 48 h later, whereas performance at 24 h was impaired by doses of 0.3 and 1.0 μg ²⁵⁴. It is possible that the higher doses of CRF, which stimulated ACTH release, might have affected performance by increasing the concentration of circulating corticosteroids, which have been shown to impair performance in this task²². I.c.v. CRF (0.00003 and 0.0003 μg) injected either immediately following the training session or 1 h prior to testing, impaired performance 24 h after training²⁵⁴. Sahgal et al.²⁰³ observed a similar impairment of passive avoidance performance by i.c.v. CRF (0.1 μg) when injected immediately post-training or 1 h prior to testing. When tested 48 h post-training 0.03 μg i.c.v. CRF injected 1 h prior to testing improved performance²⁵⁴. In contrast, 0.1 μg CRF injected into the amygdala immediately following training enhanced performance 24 h or 1 week later^{147,138}.

TABLE II

Effects of hypophysectomy, adrenalectomy, or dexamethasone treatment on the responses to intracerebral CRF administration

'+' indicates a CRF-induced increase in the measure; '-' a decrease. '0' indicates that the treatment does not alter the response to CRF; 'B' indicates that it blocks the response.

Measure	CRF effect	Hypox effect	Adrex effect	Dex effect	Refs.
Physiological					
Plasma glucose	+	0	0		38
Arterial pressure	+	0	0	0	85
Heart rate	+	0	0	0	85
Gastrointestinal function:					
Gastric acid secretion	-	0	B		236
			B		66
Gastric emptying	-	0	0		143
			0		238
Small bowel transit	-	0			143
Large bowel transit	+	0			143
Behavioral					
Locomotor activity	+	0		0	27,32
					75
Ingestion	-	0			163
				0	27
Grooming	+	0		0	163
				0	27
				0	74
Geller-Seifert test	-			0	32
Exploration (MCC)	-	0			20
Active avoidance	+	0	0		254

5.11. Primate behaviors

There have been relatively few studies of the behavioral effect of CRF in primates. When administered i.v. to chair-restrained rhesus monkeys, 10 and 125 $\mu\text{g/kg}$ CRF increased struggling and investigatory behaviors¹²⁰. When tested in their home cages, both doses increased vocalizations, threatening behavior and the time spent passively lying down. An increase in ingestive and self-directed behaviors was observed at the lower dose only, whereas decreases in environmental exploration, grooming and huddling behaviors were observed at the 125 $\mu\text{g/kg}$ dose. I.c.v. CRF (20 and 180 μg) increased the general activity in chair-restrained rhesus monkeys, but did not significantly affect any specific behavior¹²¹. In the home cage, both doses of CRF increased vocalizations. At the higher dose an increase in huddling and lying down behavior was observed. CRF (10 μg) administered i.c.v. to infants separated from their mothers, inhibited behavior¹²³. Lower doses did not produce observable changes. Although these behavioral changes were accom-

panied by increases in plasma CRF, ACTH and cortisol, they were not mimicked by peripheral CRF administration that resulted in higher circulating concentrations of all 3 hormones¹²³.

In squirrel monkeys, i.c.v. CRF had complex effects; generally, CRF increased measures of activation and vigilance²⁶¹. CRF (10 μg) increased locomotor activity under several conditions, an effect that was prevented by ahCRF (10 μg i.c.v.). Vigilance (change of gaze) was enhanced by 0.1 or 1.0, but not by 10 μg CRF. Interestingly, 10 μg ahCRF had a similar effect, suggesting that this antagonist has partial agonist properties. The authors considered that the effects of the lower doses of CRF corresponded to increased vigilance, whereas higher doses increased escape and withdrawal²⁶¹.

6. CRF AND THE IMMUNE SYSTEM

CRF has been reported to have both direct and indirect effects on the immune system. In rats, i.c.v.

TABLE III

Effects of naloxone on the response to CRF

BEA means β -endorphin antiserum. B indicates that it blocks the effect of CRF; Att, attenuates the effect of CRF; 0, does not alter response to CRF; ?, the dose of CRF for this experiment was not explicitly stated in the report.

Measure	CRF dose	Effect of naloxone	Effect of BEA	Effect of opiates	Refs.
Endocrine					
LH secretion	10 μg	0			188
	10 μg		B		182
	10 μg	Att			6
LH and FSH secretion	100 $\mu\text{g/h IV}$	B			90 (monkey)
	100 $\mu\text{g/h IV}$	B			13 (woman)
Physiological					
Blood pressure increase	8 μg	B			207
Tachycardia	8 μg	B			207
Bradycardia	10 $\mu\text{g IV}$	B			130
Gastrointestinal function:					
Gastric acid secretion	10 $\mu\text{g?}$	0			236
	12 μg	0			66
	12 μg	Att			142
Gastric emptying	1 μg	0			238
	0.6 μg	B			143
Small bowel transit	6 μg	B			143
Large bowel transit	0.6 μg	0			143
Epileptic		B			153
Neurochemical					
DA release	1 μg	0			69
NE release	1 μg	0			69
Behavioral					
Locomotor	1 μg	0			132
	8 μg	B			207
Grooming	1 μg	B			74
	8 μg	B			207
Female sexual behavior (lordosis)	2 μg	B	B		218
	0.5 μg	Att	Att		219
Male sexual behaviors	4 μg	B			220
Exploratory behavior	0.05 μg	B			16

injection of CRF (0.1–1 μ g) dose-dependently reduced splenic natural killer (NK) cell cytotoxicity measured 1 h after injection, with a minimum effective dose of 1 μ g¹¹⁷. No such effect was observed with peripherally administered CRF (5, 10 or 20 μ g/kg) nor with CRF (10^{-12} – 10^{-6}) in vitro. The effect of i.c.v. CRF was antagonized by i.c.v. (100 μ g) but not peripherally administered ahCRF (0.5 mg/kg). In a subsequent study, the decreased NK cytotoxicity induced by i.c.v. CRF (1 μ g) was found to be paralleled by an increase in plasma NE and both effects were abolished by pretreatment (1 h) of the rats with chlorisondamine (3 mg/kg)¹¹⁸. However, the CRF-induced elevations of plasma ACTH and corticosterone were not altered by chlorisondamine, suggesting that the effect of i.c.v. CRF was mediated by the autonomic nervous system and not by ACTH or corticosterone. The depressant effect of repeated footshock treatment on NK cell activity was prevented by i.c.v. injection of antibody to CRF, suggesting that cerebral secretion of CRF mediates this effect of footshock¹¹⁹.

Other workers have focussed on direct effects of CRF

on lymphocytes. Webster and De Souza²⁵⁵ found binding sites for CRF in mouse spleen, resembling those found in the pituitary. The binding sites were localized primarily in the red pulp and marginal zones and were not found in the periaarteriole and peripheral follicular white pulp regions. The authors consider that this distribution suggests the absence of binding sites on B and T cells, consistent with their own observations of a lack of CRF binding in human peripheral blood lymphocytes and mouse thymus. The binding sites appear to be on splenic macrophages²⁵⁶. These results are partially consistent with those of Singh and Fudenberg who reported CRF binding sites on monocytes and to a lesser extent T cells from human donors²¹⁷.

McGillis et al.¹⁵⁷ reported that incubation of rat B lymphocytes with CRF (10^{-10} – 10^{-7} M) in vitro stimulated proliferation. Interestingly, ahCRF was as effective as CRF. Consistent with this, CRF (10^{-10} – 10^{-8} M) in vitro enhanced the ability of concanavalin A and phytohemagglutinin, but not pokeweed mitogen, to stimulate human lymphocyte proliferation and 10^{-9} M CRF increased

TABLE IV

Effects of CRF antagonists on responses in stress

B = blocked; 0 = no significant effect; Att = the effect was attenuated; ? = the dose was not specified.

Measure	Stressor	ahCRF dose	ahCRF effect	CRF antibody	Refs.
Endocrine					
Plasma ACTH	ether	1 mg IV	B	B	193, 194
	ether			B	177
	ether			B	164
	cold swims		B		164
	immobilization		B		134
	formalin			B	150
	restraint			B	150
Plasma GH	foot shock	100 μ g	B		191
	ether			B	177
Plasma LH	foot shock	100 μ g	B		195
	ether	100 μ g		0	177
Physiological					
Plasma NE	ether	100 μ g	0		39
Plasma EPI	ether	100 μ g	B		39
Gastrointestinal function:					
Gastric acid secretion	surgery	10, 50 μ g	B		227
	partial restraint	5–50 μ g	B		144
Gastric emptying	surgery	?	B		239
	partial restraint	60 μ g	B		144
	noise	200 ng	B	B (IP)	98
Bowel transit	partial restraint	50 μ g	B		258
	partial restraint	50 μ g	B		144
Behavioral					
Ingestion	restraint	50 μ g	Att		134
Shock-induced freezing	foot shock	25 μ g	B		124
Shock-induced fighting	shock	5, 25 μ g	B		242
Exploratory behavior in the MCC	restraint	10–50 μ g	B		17
Defensive withdrawal	novelty	1 μ g			44, 240, 266
Conditioned emotional response		1, 5, 25 μ g	Att		52
Acoustic startle	conditioned fear	5, 25 μ g	B		234

expression of the IL-2 receptor²¹⁶. In contrast to the lack of effect of CRF in vitro on NK activity mentioned above¹¹⁷, Pawlikowski et al.¹⁸¹ found that CRF (10^{-10} – 10^{-6} M) inhibited NK activity. CRF also suppressed human peripheral leukocyte chemotaxis²²⁸.

Kavelaars et al.¹²⁸ showed that CRF ($7 \cdot 10^{-8}$ M) in vitro stimulated β -endorphin secretion by human monocytes. Interestingly, this effect appears to be mediated by interleukin-1, suggesting that this cytokine may mediate some of the other in vitro effects of CRF¹²⁸. In rats, CRF (0.1 – $10 \mu\text{g SC}$) stimulated β -endorphin production by a small proportion of splenic and mesenteric lymph node lymphocytes¹²⁹.

7. INVOLVEMENT OF THE HPA AXIS IN THE EFFECTS OF CRF

Very few of the above-described effects can be attributed to activation of the HPA axis, i.e. secondary release of ACTH, endorphins or glucocorticoids. However, this possibility has been rigorously excluded in relatively a few cases. The relevant studies are listed in Table II.

Removal of the adrenal glands did not alter the effects of i.c.v. CRF on plasma glucose³⁸, mean arterial blood pressure and heart rate⁸⁵, nor on gastric emptying^{143,238}. However, the effects of gastric acid secretion were blocked by adrenalectomy^{66,236}. This result implicates plasma catecholamines or glucocorticoids in this particular response. In terms of the behavioral responses, the effects on active avoidance behavior were not affected by adrenalectomy²⁵⁴.

In no case has hypophysectomy been shown to alter the effects of intracerebrally administered CRF. This was true for all of the physiological measures mentioned above and a wide variety of behavioral responses, including those on locomotor activity⁷⁵, feeding and grooming¹⁶³, active avoidance behavior²⁵⁴ and exploration²⁰. A functional hypophysectomy produced by dexamethasone administration to suppress pituitary secretion of ACTH did not alter the effects of i.c.v. CRF on mean arterial blood pressure and heart rate⁸⁵, locomotor activity^{27,32}, feeding²⁷, grooming^{74,163} or in the Geller-Seifter conflict test³¹.

Nevertheless, some of the effects of CRF may be due to secondary release of pituitary-adrenal hormones. Intracerebral administration of ACTH and β -endorphin induce grooming, so a secondary release of these peptide hormones within the brain cannot be excluded as a cause of this response to CRF. Indeed, the profile of the grooming response elicited by CRF closely resembles that for ACTH, suggesting that CRF-induced ACTH may be responsible for this effect⁷⁴. Also, ACTH, β -endorphin

and glucocorticoids are known to affect passive and active avoidance behavior^{22,67}, although in some cases opposite effects of the various hormones have been observed (see above).

It should also be noted that in a variety of instances, peripheral administration of CRF lacked the effects of central administration, while generally the former route of administration is more potent in activating the pituitary-adrenal system. Such instances include: LH secretion¹⁸⁸, GH secretion^{127,189}, locomotor activity²⁷, grooming behavior^{27,211}, several effects on passive and active avoidance behavior²⁵⁴ and defensive withdrawal²⁴⁰. However, many of the gastrointestinal effects of CRF can be elicited by peripheral administration: gastric acid secretion^{131,144,237}; gastric emptying^{143,144,238,260}; increased large bowel transit^{144,258}; migrating motor complexes⁹⁹ and antral activity⁸⁸.

8. INVOLVEMENT OF THE AUTONOMIC NERVOUS SYSTEM IN THE EFFECTS OF CRF

As reviewed above, i.c.v. CRF administration can activate the sympathetic nervous system. In general this occurs only at relatively high doses of CRF ($\geq 1 \mu\text{g}$). Thus there is a real possibility that responses observed at doses of CRF in this range may be due to sympathetic activation. Brown and Fisher showed that the ganglionic blocker, chlorisondamine, reversed or markedly attenuated the effects of i.c.v. CRF on the increases in plasma concentrations of NE, EPI and glucose³⁸ and those on heart rate and blood pressure^{84,85}. Autonomic activation seems very likely to account for many of the gastrointestinal responses to i.c.v. CRF (gastric acid secretion, gastric emptying and gastrointestinal motility, see above) and may also account for some behavioral responses. The apparent mediation of the pyrogenic effects of IL- 1β by CRF activation of the sympathetic nervous system^{43,199} was also discussed above.

Britton and Indyk²⁸ investigated the possibility that the activation of the autonomic nervous system contributes to the locomotor-activating effects of CRF, using the ganglionic blockers chlorisondamine and hexamethonium. In the home cage, chlorisondamine but not hexamethonium, attenuated the CRF-induced (0.4 and $0.8 \mu\text{g}$) increase in locomotion. In an open field, the CRF-induced decrease in locomotion and the increase in grooming were not affected by chlorisondamine but were attenuated by hexamethonium. Thus, these results suggest that an activation of the ANS could contribute to the actions of CRF on locomotor activity. By contrast, the CRF-induced decrease in food consumption was not significantly antagonized by either drug in either testing environment (except by chlorisondamine tested in the

home cage with $0.4 \mu\text{g}$ CRF). Thus, autonomic effects of CRF are not likely to contribute significantly to the ability of CRF to inhibit ingestive behavior. Neither hexamethonium nor chlorisondamine blocked the effect of CRF on the acoustic startle response¹⁴⁸. However, both drugs tended to inhibit the CRF-induced increase in startle. The ability of i.c.v. CRF ($1.0 \mu\text{g}$) to diminish NK cell activity in the rat was abolished by chlorisondamine in parallel with its ability to prevent the increase in plasma NE¹¹⁸.

The parasympathetic nervous system may also be involved in the effects of i.c.v. CRF. Several findings suggest that the vagus nerve plays a role in the effects of i.c.v. CRF on gastrointestinal functions (see above). Also, Fisher³³ reported that the i.c.v. CRF-induced change in the baroreflex control of heart rate was attenuated by atropine methyl nitrate, implicating a role for the parasympathetic nervous system (presumably the vagus) in this response. Thus the involvement of the autonomic system in the behavioral responses to i.c.v. CRF needs to be investigated further.

9. SITES OF ACTION OF CRF WITHIN THE BRAIN

Relatively little is known concerning where CRF acts within the brain to affect the variety of responses discussed above. Brown³⁴ studied the site(s) of action of the effects of CRF on plasma NE by injecting CRF ($1 \mu\text{g}$) into 50 different brain tissue sites. Some sites were responsive to CRF and some were not. When responses occurred at particular sites, they were not substantially greater than those obtained after intraventricular injections. A possible explanation is that the site of action of CRF is periventricular and that the tissue injections leak into the cerebral ventricular system.

The locomotor-activating effect of CRF ($0.5 \mu\text{g}$) injected into the substantia innominata/lateral preoptic region was significantly greater than that observed following injections of CRF into the pedunculopontine nucleus or frontal cortex²⁴³. An intermediate effect of CRF was observed when injected into the nucleus accumbens or the central nucleus of the amygdala²⁴³. However, in another study, injection of CRF ($0.1 \mu\text{g}$) into the amygdala of rats decreased locomotor activity in an open field¹⁴⁷. Injection of CRF ($0.06 \mu\text{g}$) into the VTA increased locomotor activity in a photocell cage¹²⁵. A similar effect was observed following higher doses i.c.v. ($2\text{--}20 \mu\text{g}$). In an open field, i.c.v. CRF ($2 \mu\text{g}$) increased the latency to move, but had no effect on locomotor activity, whereas $2 \mu\text{g}$ injected intra-VTA had no effect on latency to move but increased locomotor activity. It was concluded that the action of CRF on locomotor activity was not exerted in the VTA.

Injection of CRF ($0.1 \mu\text{g}$) into the amygdala of rats decreased locomotor activity in an open field and enhanced performance in the passive avoidance test^{138, 147}. However, because the effect of CRF injected into other sites within the brain was not examined, we cannot be certain that the amygdala was the active site or whether the CRF diffused from the amygdala to other brain sites. In a subsequent study in mice, Lee and Tsai¹³⁹ compared the locomotor-activating effects of CRF injected into the amygdala ($0.02 \mu\text{g}$ each side), the dentate gyrus of the hippocampus ($0.01 \mu\text{g}$) and the caudate nucleus ($0.05 \mu\text{g}$). Injections into the caudate nucleus were ineffective, while the hippocampal injections were more potent than those in the amygdala. CRF ($0.1 \mu\text{g}$) injected into the amygdala immediately following passive avoidance training in rats increased retention 24 h and 1 week later¹³⁸. A lower dose ($0.01 \mu\text{g}$) also facilitated retention in a sexually motivated appetitive test 24 h and 1 week post-training, but $0.1 \mu\text{g}$ impaired performance. Thus, although CRF injected into the amygdala affects performance in memory tests, there appears to be a difference in the sensitivity between appetitively and aversively motivated behaviors. Amygdaloid lesions impaired the CRF-induced increment in the startle response¹⁴⁸, but CRF injected into the amygdala did not alter the response¹⁴⁸, although injections of CRF into the parabrachial nucleus did (M. Davis, personal communication).

In an attempt to localize the anorectic effects of CRF, $0.5 \mu\text{g}$ of the peptide was injected into the PVN, lateral hypothalamus, ventromedial hypothalamus, globus pallidum or striatum of rats¹³⁶. The only effective location was the PVN, in which CRF also enhanced grooming and movement. Injections of CRF into the mesencephalic gray, arcuate-ventromedial hypothalamus or the medial preoptic area inhibited lordosis behavior^{218, 219, 223}.

Two groups of investigators have used the cold cream blocking technique to determine where within the ventricular system CRF acts. Tazi et al.²⁴³ found that $1 \mu\text{g}$ of CRF had a locomotor activating effect when injected either into the lateral ventricles or the cisterna magna. However, when the cerebral aqueduct was blocked by injection of cold cream, the latter site was no longer effective. These data in conjunction with their data on localized injections (see above) led the authors to conclude that the locomotor activating effect of CRF in a familiar environment involves an action of CRF in the ventral forebrain. We have investigated the effect of a similar block of the cerebral aqueduct on the ability of CRF ($0.02 \mu\text{g}$) to decrease exploratory behavior in the MCC. Blockade of the cerebral aqueduct prevented the decrease in exploration when CRF was injected into the 4th ventricle, but not the lateral ventricles²²⁶. Further,

blocking access of the peptide to the anteroventral quadrant of the third ventricle (AV3V) blocked the effect of lateral ventricle injection of CRF²²⁶. When other areas on the third ventricular surface (but not the AV3V) were blocked, the effect of CRF was not significantly attenuated. It is of interest that this region of the third ventricle was also determined to be a critical site in the ability of angiotensin to increase drinking¹¹⁰, bradykinin to increase blood pressure¹⁴⁶ and ACTH to increase grooming⁷¹.

10. INTERACTION OF CRF WITH OTHER NEUROTRANSMITTER SYSTEMS

Relatively little is known of the neurotransmitter systems with which CRF interacts to effect the responses described above.

10.1. Catecholamines

Van Loon et al. first investigated the effect of CRF on cerebral catecholamine metabolism in rats²⁵². Using *m*-hydroxybenzylhydrazine to inhibit *L*-aromatic amino acid decarboxylase (a synthetic enzyme for catecholamines and serotonin (5-HT)), they failed to find any effects of IC CRF (5 μ g) on the synthesis of 3,4-dihydroxyphenylalanine (a precursor of dopamine, DA and NE) or 5-hydroxytryptophan (a precursor of 5-HT). Likewise, using the monoamine oxidase inhibitor, pargyline, to prevent degradation of catecholamines, CRF (20 μ g IC) failed to alter the increased accumulation of DA, NE, EPI or 5-HT. They concluded that CRF did not alter the 'turnover' of cerebral catecholamines. However, Andersson et al.⁷ studying the effects of i.v. CRF (100 μ g/kg) in hypophysectomized rats found that the disappearance of NE (determined by histofluorescence) from the median eminence region was accelerated following inhibition of synthesis with α -methyl-*p*-tyrosine. Moreover, the disappearance of NE from the PVN was decreased. They concluded that the changes reflected regulatory feedback mechanisms and that NE most likely had a stimulatory effect on CRF-containing neurons in the PVN.

We have studied the effects of i.c.v.-administered CRF on the cerebral concentrations of catecholamines, indoleamines and their catabolites. CRF was administered into the lateral ventricles of mice and various brain regions sampled 30 min later. One μ g of CRF significantly increased the concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC, a catabolite of DA) in the prefrontal cortex, nucleus accumbens, septum, striatum, hypothalamus and brain stem and of 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG, a catabolite of NE) in the prefrontal cortex, hypothalamus and brain-

stem of mice, without any significant changes in the parent catecholamines⁶⁹. A lower dose of CRF (0.2 μ g) had some of the same effects. Rather similar effects were observed using SC CRF administration at higher doses (1 or 10 μ g); DOPAC was increased in prefrontal cortex and MHPG in prefrontal cortex, hypothalamus and brainstem. These effects of i.c.v. and SC administration of CRF resemble those observed with behavioral stressors such as footshock or restraint⁶⁸, except that no increases in brain tryptophan or in 5-HT metabolism were observed. The effects of i.c.v. CRF on DOPAC and MHPG were not altered by naloxone pretreatment (0.8 mg/kg)⁶⁹. A recent report found very similar results in rats¹⁵⁶. One μ g CRF increased DOPAC:DA ratios in frontal cortex, nucleus accumbens, striatum and amygdala and 10 μ g had similar effects in all these regions plus the hippocampus. MHPG:NE ratios were increased by both doses of CRF in frontal cortex and hippocampus.

Other authors have essentially confirmed CRF-induced increases in DA and NE metabolism in rats. Kalivas et al.¹²⁵ observed increases in DOPAC and homovanillic acid (HVA, another DA catabolite) in the prefrontal cortex, nucleus accumbens and striatum following i.c.v. administration of CRF (2 or 20 μ g), although these effects were only statistically significant at the higher dose. By contrast, decreases in DOPAC and HVA were observed in the prefrontal cortex of rats following CRF administration (0.2 or 2.0 μ g) into the VTA. Butler et al. found that 1 μ g CRF injected into the locus coeruleus increased cerebral concentrations of 3,4-dihydroxyphenylethyleneglycol (DHPG, another catabolite of NE) in the amygdala and posterior hypothalamus 45 min later⁴⁴. The minimum effective dose of CRF i.c.v. was 0.5 μ g. However, similar changes were observed with lower doses of CRF (minimum effective dose 0.01 μ g) when the peptide was applied locally in the region of the LC. The effects of i.c.v. CRF on MHPG and DHPG are consistent with the increased firing rate of LC neurons observed following i.c.v. administration of 1 μ g CRF²⁵⁰ (see above). The activation of cerebral biogenic amines has been confirmed in pigeons in which i.c.v. CRF (5.6–30 μ g/kg) increased CSF concentrations of DOPAC and HVA, with lesser effects on MHPG¹⁴.

The association of CRF administration with an activation of catecholamine metabolism is also consistent with reports that striatal tyrosine hydroxylase from rats or mice is activated by CRF (10^{-8} – 10^{-6} M) in vitro^{172,173}.

We know that the release of CRF occurs during stress¹⁸⁷ and that catecholaminergic systems are activated under similar conditions⁷². It has long been known that NE plays a role in the regulation of CRF release²⁵⁶, but the nature of this role has been confused. Anatomical studies have established that there is a direct noradren-

ergic input to the hypothalamic PVN⁵⁷. A recent study provides good evidence for a dopaminergic input also¹⁵¹. Other studies have suggested catecholaminergic input to CRF-containing cells in other regions: the central nucleus of the amygdala, the bed nucleus of stria terminalis, but not the suprachiasmatic nucleus¹¹³. Recent evidence from several laboratories suggest that NE is largely stimulatory on CRF release, and that during stress noradrenergic terminals in the PVN stimulate CRF release through an α_1 -receptor^{4,184,235}. Al-Damluji³ and Plotsky et al.¹⁸⁵ have reviewed the available evidence and provided plausible explanations of the previous interpretations.

This leaves us with the interesting situation that CRF can both activate and be activated by noradrenergic systems. Such a reciprocal relationship should perhaps not be surprising, considering that adapting to stress (i.e. coping) is a most important function for any living organism. It should perhaps be noted, however, that the activation of cerebral catecholaminergic systems occurs only at relatively high doses of CRF, higher than the minimal doses required to elicit several behavioral effects.

Although CRF may alter DA release, dopamine systems do not appear to be involved in the locomotor-activating effect of CRF observed in a familiar environment because α -flupenthixol (a dopamine receptor antagonist) did not antagonize the effect of 1 μ g i.c.v. CRF except at cataleptic doses¹³². Likewise, haloperidol failed to reverse the effects of intra-VTA injections of CRF on locomotor activity¹²⁵. Further, 6-hydroxydopamine lesions of the nucleus accumbens, which prevented the locomotor-activating effects of amphetamine, had no effect on the CRF-induced increase in locomotor activity²³². Nevertheless, CRF (0.02 or 0.1 μ g) potentiated amphetamine-induced stereotyped behavior in rats⁵¹.

Noradrenergic systems might have a role in mediating the locomotor activating effect of CRF observed in a familiar environment. Chronic treatment with desmethyl-imipramine, a norepinephrine re-uptake inhibitor, enhanced the locomotor-activating effect of CRF⁷⁹. Yohimbine, an α_2 -antagonist (10 nmol) administered i.c.v. and phentolamine, an α -antagonist, (10 nmol) inhibited the CRF-induced increase in locomotor activity without affecting caffeine-induced increases in activity¹¹⁴.

The β -adrenergic antagonist, propranolol, enhanced the CRF-induced increase in locomotor activity in a familiar testing environment⁵⁰. In a conditioned emotional response, *l*-propranolol (but not *d*-propranolol) blocked the CRF-induced increased suppression of responding in the conditioned-stimulus, but not the pre-conditioned stimulus components⁵⁰. This could indicate that β -adrenoceptors regulate stress-related behavioral responding. *l*-Propranolol (but not the *d*-form) reversed

the restraint- and CRF-induced increases in defensive withdrawal behavior in rats²⁶⁶. This effect appears to be mediated by a central β_1 -adrenoceptor²⁶⁵. Propranolol also blocked the CRF-induced decrease in the pentobarbital-induced sleeping time¹¹⁵.

The effect of restraint on exploratory behavior of mice in the MCC appears to involve a NE-stimulated release of CRF¹⁹. Treatments that increase NE release including restraint¹⁶ and the α_2 -antagonist, idazoxan (1 mg/kg), decreased exploratory behavior^{18,19}. In contrast, the α_2 -agonist (clonidine 25 μ g/kg, IP) or the noradrenergic-selective neurotoxin, DSP-4, increased exploration in unrestrained mice and antagonized the restraint-induced decrease in this response¹⁹. When combined, DSP-4 and clonidine completely blocked the effect of restraint stress¹⁹. The α_1 -receptor-antagonist, prazosin (200 μ g/kg), also prevented the behavioral effect of restraint, whereas the α_1 -agonist, phenylephrine (50 or 100 ng i.c.v.) decreased exploratory behavior¹⁹. Because phenylephrine does not cross the blood-brain barrier readily and the dose administered in these studies was significantly lower than that at which cardiovascular effects are observed when administered peripherally, it is probable that central noradrenergic receptors are involved in these effects of phenylephrine. None of the above treatments consistently affected locomotor activity. Neither DSP-4 nor prazosin altered the CRF-induced decrease in exploration. However, the CRF-antagonist, ahCRF (20 μ g), reversed the phenylephrine-induced decrease in exploratory behavior¹⁹. Rather similar results were obtained studying defensive withdrawal in rats. Prazosin or clonidine decrease defensive withdrawal in naive rats²⁶⁶. Restraint increased defensive withdrawal in rats familiar with the apparatus and this effect was prevented by prazosin or clonidine²⁶⁶. I.c.v. CRF also increased defensive withdrawal^{44,240,266}. I.c.v. phenylephrine mimicked the effect of restraint or CRF²⁶⁶. Prazosin reversed the effect of phenylephrine but not CRF²⁶⁶. However, the effect of phenylephrine was reversed by i.c.v. ahCRF²⁶⁶. Thus in both these paradigms (exploratory behavior in mice and defensive withdrawal in rats) noradrenergic and CRF systems appear to interact such that noradrenergic systems regulate the release of endogenous CRF via an α_1 -receptor. Such an arrangement parallels the organization of the noradrenergic and CRF systems instrumental in ACTH release^{3,184,235}.

10.2. Serotonin

In the studies with CRF discussed above, Van Loon did not find any changes of 5-HT metabolism related to i.c.v. injection of CRF²⁵² and we did not observe any changes in 5-HT or its catabolite, 5-hydroxyindoleacetic acid (5-HIAA) after i.c.v. infusion of 0.2 or 1.0 μ g⁶⁹.

However, i.c.v. CRF (5.6–30 $\mu\text{g/kg}$) increased CSF concentrations of 5-HIAA in pigeons¹⁴. Moreover, a recent report indicated that 3 μg CRF administered i.c.v. to rats activated tryptophan hydroxylase assayed in vitro²¹⁴. This effect of CRF was also obtained with infusions of CRF into the central amygdaloid nucleus²¹⁵. Notably these effects were observed only at very high doses of CRF, so their physiological significance is unclear.

10.3. Acetylcholine

When injected together with carbachol (4 μg) into the medial frontal cortex in rats, CRF (0.001–0.01 μg) inhibited the carbachol-induced repetitive forepaw treading ('boxing')⁵⁵. This result suggests that CRF may have anticholinergic properties. A higher dose of CRF (0.2 μg) injected alone into this region had no observable effects on behavior.

10.4. GABA

The effects of benzodiazepines on the responses to CRF (see above) provide indirect evidence for an interaction between CRF and GABAergic systems, but no direct evidence for an interaction between CRF and benzodiazepine receptors or benzodiazepines and CRF-receptors has been reported. Nevertheless, Sirinathsinghji and Heavens²²¹ found that CRF injected into the striatum and globus pallidum stimulated GABA release in vivo.

10.5. Endogenous opiates

A recent study found extensive colocalization of enkephalin- and irCRF in all regions of the PVN, the medial preoptic area, bed nucleus of the stria terminalis, periventricular hypothalamic nucleus, lateral and dorsal hypothalamic areas and subincertal nucleus²⁰⁶. Input of cells containing irACTH (which may thus also contain β -endorphin) to irCRF cells also occurs in the PVN and the bed nucleus of the stria terminalis¹¹³. CRF has been shown to possess a powerful ability to release endorphins both in vivo and in vitro. Sirinathsinghji et al.²²² showed that 10^{-12} – 10^{-8} M CRF applied in 75 min pulses stimulated the release of both dynorphin and Met-enkephalin from striatal slices. Moreover, similar concentrations of CRF stimulated the release of both peptides from the caudate nucleus and globus pallidus in vivo. Both the in vivo and the in vitro effects were prevented by ahCRF (10^{-6} M). Thus some of the effects of CRF administration may be mediated by endogenous opiates.

The involvement of endogenous opiates in physiological and behavioral responses is normally determined by testing the sensitivity to the opiate antagonist, naloxone or its longer acting analog, naltrexone. However, more

specificity can be obtained by the use of antisera to the various endogenous opioid peptides and this has been done in a few studies with CRF. The data on the ability of naloxone, naltrexone or β -endorphin antisera to antagonize the effects on CRF are summarized in Table III.

Conaglen et al.⁵³ reported that naloxone enhanced the ACTH, cortisol and aldosterone responses to i.v. CRF administration in man. Naloxone blocked the bradycardia, but not the hypotension caused by i.v. CRF in rats¹³⁰. As discussed above, endogenous opiates are involved in other endocrine responses to CRF. Naloxone has been variously reported to reverse or attenuate the inhibitory effect of CRF on LH secretion^{6,13,90,171}, and on FSH secretion in primates^{13,90}. It also prevented the elevation of prolactin secretion²⁵³. All of these effects involve peripheral effects of CRF. According to one report, naltrexone did not alter the decrease in LH secretion caused by i.c.v. CRF¹⁸⁸. However, an attenuation of LH secretion in rats was also observed using i.c.v. antiserum to β -endorphin or dynorphin-A, but not to enkephalin¹⁸².

As mentioned above, injections of CRF into the mesencephalic gray, arcuate-ventromedial area of the hypothalamus or the medial preoptic area inhibited lordosis behavior^{218,219,223}. In the mesencephalic gray and arcuate-ventromedial area of the hypothalamus this effect of CRF was significantly inhibited or abolished by pretreatment of the tissue site with naloxone or antisera to β -endorphin, but not by antisera to dynorphin or Met-enkephalin^{218,223}. In the medial preoptic area, CRF appears to act synergistically with β -endorphin to inhibit lordosis²¹⁹. In both the mesencephalic gray and medial preoptic area the effect of CRF could be abolished with infusions of LHRH into these regions, whereas the facilitation of lordosis observed with anti- β -endorphin and CRF antisera were blocked by an LHRH antagonist. Thus, in these two regions CRF appears to inhibit lordosis by inhibiting the release of LHRH^{218,219,223}. In male rats, naloxone infused into the third ventricle (10 μg) blocked the disruptive effect of CRF on sexual behavior²²⁰.

In addition to that observed with sexual behavior, opiates might also be involved in some of the other behavioral effects of CRF. Naloxone reversed the CRF-induced decrease in exploratory behavior in mice at a dose that had no significant effect on this response in the absence of CRF¹⁶. Naloxone also blocked the i.c.v. CRF-induced increase in grooming behavior in rats⁷⁴. However, naloxone (0.02–5.0 mg/kg) has been reported not to block the locomotor-activating effect of CRF^{132,153}, although, in a third study naloxone did block this effect of CRF²⁰⁷. The dose of naloxone used in the latter

study (presumably 3 mg/kg), could be considered high and the authors failed to exclude possible sedative effects of naloxone.

11. THE ROLE OF CRF IN STRESS-RELATED EFFECTS

The above review has detailed a large number of endocrine, neurochemical, electrophysiological and behavioral changes elicited by CRF. As pointed out earlier most of these mimic or are compatible with responses observed in stress^{93,133}. Of critical significance are the activation of the pituitary-adrenal system, the sympathetic nervous system and adrenal medulla and cerebral catecholamines, all of which are regarded as primary components in the stress response^{12,72}. Nevertheless, these results themselves could be interpreted to indicate that i.c.v. administration of CRF is stressful and thus elicits a range of responses commonly observed in stress. A crucial test is the ability of a CRF antagonist to prevent the changes observed in stressful situations. Fortunately, both CRF antisera and at least one peptide antagonist of CRF exists. α -Helical CRF₉₋₄₁ appears to be relatively specific, although disappointingly high doses are needed to effectively block CRF-receptors¹⁹⁴ and because it is a peptide it does not readily cross the blood-brain barrier.

Thus far most reports have found CRF antagonists to attenuate or prevent stress-related changes (Table IV). I.c.v. ahCRF prevented the electric footshock-induced decreases in GH¹⁹¹ and LH¹⁹⁵ secretion. Antibody to CRF blocked the ether exposure-induced decreases in GH but not LH secretion¹⁷⁷. AhCRF also prevented the increase in plasma EPI, but not NE³⁹. It also prevented the gastrointestinal effects of i.c.v. CRF (gastric acid secretion, gastric emptying and gastric motility) and antibody to CRF blocked the noise-induced increase in gastric emptying⁹⁸ (see above). As far as the behavioral responses are concerned, i.c.v. ahCRF attenuated the restraint-induced decrease in feeding¹³⁴, blocked the restraint-induced decrease in exploratory behavior in the MCC¹⁷, the electric shock-induced increases in fighting²⁴² and freezing¹²², attenuated the fear-induced exacerbation of acoustic startle²³⁴ and decreased the acquisition of a CER⁵². In the defensive withdrawal task, it decreased withdrawal in a novel open field²⁴⁰ and reversed the restraint-induced increases in withdrawal in rats familiar with the apparatus²⁶⁶. These results give extraordinary cogency to the argument that CRF is an endogenous mediator of these responses.

A priori, it seems unlikely that i.c.v.-injected CRF is able to reach all cerebral CRF-receptors in sufficient concentration to activate them, but this may be the reason for the high doses of CRF necessary to elicit some of the stress-like responses. However, if, as Ono et al.¹⁷⁷

have suggested, i.c.v. CRF can activate endogenous CRF systems, there is a mechanism for a global activation of CRF receptors. An alternative possibility is that high doses of CRF specifically activate catecholaminergic systems that in turn elicit release of endogenous CRF. The uniformity of the results with the CRF antagonists is truly remarkable given the probable difficulty in obtaining adequate concentrations at the appropriate brain sites. Indeed it would not have been surprising if a variety of stress-related responses observed following CRF administration, were not affected by the antagonist. On the other hand, brain sites accessible to CRF are probably accessible to ahCRF, because the latter peptide is less hydrophilic.

A role for CRF in anxiety or stress is supported by the effects of benzodiazepines. In a number of cases, benzodiazepines elicited behavioral effects opposite to those following administration of CRF: feeding in an open field²⁴⁻²⁶; punished responding in the Geller-Seifter conflict test^{30,33}; social interaction⁷⁰; and defensive withdrawal²⁶⁶. In several cases, benzodiazepines antagonized or reversed the effects of CRF: locomotor activation by low doses of CRF in an open field¹⁴⁰; suppression of punished responding in the Geller-Seifter test^{30,33}; decreases in social interaction⁷⁰; enhancement of acoustic startle²³³; and increased defensive withdrawal²⁶⁶. Moreover, the anxiogenic benzodiazepine inverse agonist, FG 7142, like CRF, decreased punished responding in the Geller-Seifter test³³. These results suggest that CRF can be anxiogenic, so that endogenous CRF may be a mediator of anxiety. Experimentally, we are poorly equipped to distinguish anxiety from stress in animal studies.

12. CONCLUSIONS

The foregoing indicates that CRF can elicit a number of responses normally regarded as characteristic of anxiety or stress. The list includes many if not most of the responses symptomatic of stress. Because CRF antagonists are able to attenuate or reverse the effects of various stressors, CRF appears to be a mediator of these responses. Because the responses to intracerebrally administered CRF cover the entire spectrum of responses observed in stress, it is possible to postulate that the secretion of brain CRF may be both necessary and sufficient to define stress.

Postulating a role for cerebral CRF in stress suggests that the functions of CRF in the brain are akin to those involved in the activation of the HPA axis. This hypothesis is not unreasonable, but we know very little of the mechanisms regulating the release of CRF from cells outside the PVN. There is good evidence that activation

of noradrenergic systems in stress is the primary mechanism responsible for the release of CRF from neurons in the PVN, possibly through an α_1 -receptor, although undoubtedly other neurotransmitter systems (e.g. acetylcholine, 5-HT and GABA) are also involved. We do not know whether similar mechanisms operate for other CRF-containing cells in the brain. It is possible that CRF-containing neurons in the PVN have collaterals reaching to widespread areas of the brain. However, the existing anatomical evidence for CRF-containing cell bodies in extrahypothalamic regions suggests that this is not the case. An interesting alternative would be the innervation of CRF-containing cells by the network of catecholaminergic terminals in widespread areas of the brain, including the cortex. Only further detailed research on the inputs to CRF-containing neurons can resolve this issue.

Interestingly, a CRF hypothesis of stress provides support for Selye's ideas of non-specificity in stress. Selye conceived this hypothesis because he considered the sympathoadrenal and HPA responses to be common to all stressors. Although Mason¹⁵⁵ and others have criticized the non-specificity concept, it is generally agreed that both catecholamine and HPA systems are activated in most, if not all, situations commonly regarded as stressful. We conceive the primary response in stress to be the activation of cerebral noradrenergic systems, which in turn activates CRF secretion and hence activates the HPA axis. This cerebral noradrenergic activation may also be responsible for the activation of the autonomic nervous system, perhaps via descending tracts originating in the PVN. Alternatively, at high rates of CRF secretion (corresponding to high doses of CRF), CRF may directly activate the autonomic nervous system and the adrenal medulla via descending tracts. Such a hypothesis would explain the coactivation of catecholamine and CRF systems in stress and many of the data reviewed above. It may well be that combined activity of catecholamine and CRF systems is necessary to manifest all aspects of stress. For example, the direct effects of NE on cerebral neurons to increase signal-to-noise ratios may provide a mechanism for selective attention. This effect of NE would then complement independent actions of CRF, which may induce arousal or even fear.

12.1. The effect of CRF dose

The question of peptide dose is always delicate, because peptides are generally considered to be metabolically labile and because their access to specific brain sites following intracerebroventricular administration may be limited by their size¹⁴⁹. Nevertheless, because so many of the studies of intracerebrally administered CRF have been performed with lateral ventricle injections it is

permissible to make comparisons. Several effects of i.c.v. CRF exhibit biphasic responses. Thus in a novel environment, low doses of CRF increased locomotor activity ($0.01 \mu\text{g}$ ^{230,254} or $0.2 \mu\text{g}$ ¹⁴⁰), whereas high doses ($\geq 1 \mu\text{g}$) decreased it^{25,26,114,140,230,254}. Likewise, lower doses of CRF ($0.1 \mu\text{g}$) increased feeding in food-deprived rats, whereas higher doses ($5 \mu\text{g}$) decreased it⁹⁴. Also, lower doses of CRF (0.01 and $0.1 \mu\text{g}$) increased shock-induced boxing and fighting, but $1 \mu\text{g}$ disrupted the behavioral responding²⁴². In the multicompartment chamber, we observed that mice that had received doses of CRF greater than about $0.15 \mu\text{g}$ into the lateral ventricles displayed abnormal behavior, characterized by prolonged periods of inactivity. Similar effects were observed in some rats with $0.05 \mu\text{g}$ and most at $0.1 \mu\text{g}$ ²²⁶. It is possible in some circumstances that this response may reflect seizure activity which has been observed at doses in this range⁷⁷.

We suggest that the behavioral and physiological responses to i.c.v. CRF can be divided into high-dose and low-dose effects. The high-dose effects include the activation of the sympathetic nervous system and the adrenal medulla and of cerebral catecholamines. Such effects likely explain the gastrointestinal effects of i.c.v. CRF and may be associated with behavioral responses to higher doses of CRF, such as the decreased feeding and sexual behavior, the decreased locomotor activity in a novel environment, the increased locomotor activity in a familiar environment, the anxiogenic effects in the Geller-Seifter conflict test and the exacerbated acoustic startle response. On the other hand, the enhancement of locomotor behavior in a novel environment, decreased social interaction, exploratory behavior in the MCC, defensive withdrawal and increased feeding and shock-induced fighting are observed at considerably lower doses (0.005 – $0.1 \mu\text{g}$).

Curiously, both the high-dose and the low-dose effects of CRF may be specific in the sense that ahCRF has the ability to reverse the CRF-induced changes. This is true for the endocrine^{191,195} and gastrointestinal changes^{98,144,227,239,260} as well as the activation of the adrenal medulla³⁹, the increase of locomotor activity in a familiar environment and the decreased responding in the Geller-Seifter test³².

Distinctions between the low- and high-dose effects of CRF are not readily made on the basis of their pharmacological characteristics. For example, the opioid antagonist, naloxone blocked the effects of CRF on exploratory behavior¹⁶ and grooming⁷⁴. However, whereas naloxone can prevent the effects of CRF on sexual behavior²²⁰, it failed to alter the locomotor-activating effect of CRF^{132,153} and had rather complex effects on the CRF-induced changes in endocrine and gastrointestinal

functions (see Table III), all high-dose effects. The same is true for β -adrenergic antagonists. Propranolol prevented the effects of CRF on conditioned emotional responses, but enhanced the CRF-induced increase in locomotor activity in a familiar environment⁵⁰. However, propranolol reversed the effects of CRF on defensive withdrawal in rats^{265,266}.

What is the significance of the two sets of dose effects of CRF? It is possible that they represent two distinct degrees of stress. The low-dose effects may correspond to mild or moderate activations of noradrenergic systems such as may be associated with arousal and induce a state of mild anxiety. The higher doses of CRF may directly activate LC noradrenergic neurons and perhaps also dopaminergic systems. Thus the responses to high doses of CRF may reflect the combined effects of both CRF and catecholamines. The noradrenergic activation caused by high doses of CRF may provoke release of endogenous CRF, forming a positive feedback loop, escalating the release of both catecholamines and CRF. In the absence of exogenous CRF, a similar state may perhaps be achieved by prolonged or intense activation of the noradrenergic systems, such that the amounts of CRF released could be sufficient to activate catecholaminergic systems, and thus provide a positive feedback, stimulating the release of still more CRF. Such a situation may be akin to panic. It would be associated with powerful activation of both cerebral catecholaminergic systems and the autonomic nervous system, as well as cerebral CRF systems. Do two different kinds of CRF-receptor mediate the effects of low and high doses or is the difference one of access to appropriate sites? The data obtained with presently available antagonists, which can block both high- and low-dose effects, suggest the latter. However, more careful studies using a variety of CRF-antagonists may suggest the involvement of different types of receptor.

12.2. Clinical relevance

A number of observations have suggested that CRF functions abnormally in depressed patients. The response of plasma ACTH to CRF administration has been shown to be blunted^{93,111}. Subsequently, Nemeroff et al.¹⁶⁷ reported that the CSF concentrations of CRF were

significantly elevated over normals in depressed patients. This result has subsequently been confirmed by the same group¹⁶⁸. Such a result is consistent with the long standing observation of elevated plasma cortisol and an insensitivity to dexamethasone^{168,225}. More recently, depressed suicide victims have been found to exhibit a decreased number of CRF-binding sites in the prefrontal cortex¹⁶⁹. These relationships all suggest that the secretion of brain CRF is elevated in depression, and that this elevation leads to a desensitization of CRF receptors. This is not to say that depression is *caused* by an abnormality in CRF secretion. However, given the animal data reviewed above, it is not unlikely that some of the symptoms of depression may be related to the hypersecretion of CRF.

The link between noradrenergic systems and CRF discerned for exploratory behavior in the MCC and possibly in defensive withdrawal, is remarkable in the context of depression. For years the catecholamine hypothesis of depression has dominated experimentation in biological psychiatry. Recently, the hypothesis has been inverted, and it is now believed that depressed patients may exhibit hypersecretion of NE rather than the hyposecretion originally postulated. This position is reinforced by data from animal models of depression²⁵⁷. Again, it is not necessarily true that a hyperactivity of noradrenergic systems *causes* depression, but a CRF-noradrenergic interaction might be involved. The concept that hyperactivity in cerebral noradrenergic systems may stimulate hypersecretion of CRF reconciles the two hypotheses. In other words, the new catecholamine and the CRF hypotheses are not distinct, but merely reflect sequential steps in the same chain. If this is true, therapies for depression may be based not only on forcing down-regulation of noradrenergic systems, but also on antagonism of CRF.

Acknowledgements. The research from our laboratory reported in this review was supported by the National Institute of Mental Health (MH25486), the National Institute of Neurological Disease and Stroke (NS27283) and a fellowship to CWB (MH09680). We are grateful to Lionel Bueno and Gary Glavin for suggestions on sections of the manuscript and to Dawn Britt for assistance in preparing the manuscript.

REFERENCES

- 1 Aguila, M.C. and McCann, S.M., The influence of hGRF, CRF, TRH and LHRH on SRIF release from median eminence fragments, *Brain Res.*, 348 (1985) 180-182.
- 2 Aguilera, G., Wynn, P.C., Harwood, J.P., Hauger, R.L., Millan, M.A., Grewe, C. and Catt, K.J., Receptor-mediated actions of corticotropin-releasing factor in pituitary gland and nervous system, *Neuroendocrinology*, 43 (1986) 79-88.
- 3 Al-Damluji, S., Adrenergic mechanisms in the control of corticotrophin secretion, *J. Endocrinol.*, 119 (1988) 5-14.
- 4 Al-Damluji, S., Perry, L., Tomlin, S., Bouloux, P., Grossman, A., Rees, L.H. and Besser, G. M., Alpha-adrenergic stimulation of corticotropin secretion by a specific central mechanism in man, *Neuroendocrinology*, 45 (1987) 68-76.
- 5 Aldenhoff, J.B., Gruol, D., Rivier, J., Vale, W. and Siggins, G., Corticotropin-releasing factor decreases post-burst hyperpolarizations and excites hippocampal neurons in vitro, *Science*, 221 (1983) 875-877.
- 6 Almeida, O.F.X., Nikolarakis, K.E. and Lennon, A.M.,

- Evidence for the involvement of endogenous opioids in the inhibition of luteinizing hormone by corticotropin-releasing factor, *Endocrinology*, 122 (1988) 1034-1041.
- 7 Andersson, K., Agnati, L.F., Fuxe, K., Eneroth, P., Harstrand, A. and Benfenati, F., Corticotropin-releasing factor increases noradrenaline turnover in the median eminence and reduces noradrenaline turnover in the paraventricular region of the hypophysectomized male rat, *Acta Physiol. Scand.*, 120 (1984) 621-624.
 - 8 Antoni, F.A., Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of 41-residue corticotropin-releasing factor, *Endocrine Rev.*, 7 (1986) 351-378.
 - 9 Arase, K., York, D.A., Shimizu, H., Shargill, N. and Bray, G.A., Effects of corticotropin-releasing factor on food intake and brown adipose tissue thermogenesis in rats, *Am. J. Physiol.*, 255 (1988) E255-E259.
 - 10 Arnsten, A.T. and Segal, D.S., Naloxone alters locomotion and interaction with environmental stimuli, *Life Sci.*, 25 (1979) 1035-1042.
 - 11 Arnsten, A.F.T., Berridge, C.W. and Segal, D.S., Stress produces opioid-like effects on investigatory behavior, *Pharmacol. Biochem. Behav.*, 22 (1985) 803-809.
 - 12 Axelrod, J. and Reisine, T.D., Stress hormones: their interaction and regulation, *Science*, 224 (1984) 452-459.
 - 13 Barbarino, A., De Marinis, L., Tofani, A., Della Casa, S., D'Amico, C., Mancini, A., Corsello, S. M., Sciuto, R. and Barini, A., Corticotropin-releasing hormone inhibition of gonadotropin release and the effect of opioid blockade, *J. Clin. Endocrinol. Metab.*, 68 (1989) 523-528.
 - 14 Barrett, J.E., Zhang, L., Ahlers, S.T. and Wojnicki, F.H., Acute and chronic effects of corticotropin-releasing factor on schedule-controlled responding and neurochemistry of pigeons, *J. Pharmacol. Exp. Ther.*, 250 (1989) 788-794.
 - 15 Berkenbosch, F., Schipper, J. and Tilders, F.J.H., Corticotropin-releasing factor immunostaining in the rat spinal cord and medulla oblongata: an unexpected form of cross-reactivity with substance P, *Brain Res.*, 399 (1986) 87-96.
 - 16 Berridge, C.W. and Dunn, A.J., Corticotropin-releasing factor elicits naloxone-sensitive stress-like alterations in exploratory behavior in mice, *Regulat. Pept.*, 16 (1986) 83-93.
 - 17 Berridge, C.W. and Dunn, A.J., A corticotropin-releasing factor antagonist reverses the stress-induced changes of exploratory behavior in mice, *Harm. Behav.*, 21 (1987) 393-401.
 - 18 Berridge, C.W. and Dunn, A.J., α_2 -Noradrenergic agonists and antagonists alter exploratory behavior in mice, *Neurosci. Res. Commun.*, 1 (1987) 97-103.
 - 19 Berridge, C.W. and Dunn, A.J., Restraint-stress-induced changes in exploratory behavior appear to be mediated by norepinephrine-stimulated release of CRF, *J. Neurosci.*, 9 (1989) 3513-3521.
 - 20 Berridge, C.W. and Dunn, A.J., CRF and restraint-stress decrease exploratory behavior in hypophysectomized mice, *Pharmacol. Biochem. Behav.*, 34 (1989) 517-519.
 - 21 Bissette, G., Reynolds, G.P., Kilts, C.D., Widerlöv, E. and Nemeroff, C.B., Corticotropin-releasing factor-like immunoreactivity in senile dementia of the Alzheimer type, *JAMA*, 254 (1985) 3067-3069.
 - 22 Bohus, B., De Kloet, E.R. and Veldhuis, H.D., Adrenal steroids and behavioral adaptation: relationship to brain corticoid receptors. In D.D. Ganten and D. Gash (Eds.), *Adrenal Actions on Brain*, Springer, Berlin, 1982, pp. 107-148.
 - 23 Bolles, R.C., Species-specific defense reactions and avoidance learning, *Psychol. Rev.*, 77 (1970) 32-48.
 - 24 Britton, D.R. and Britton, K.T., A sensitive open field measure of anxiolytic drug activity, *Pharmacol. Biochem. Behav.*, 15 (1981) 577-582.
 - 25 Britton, D.R., Koob, G.F., Rivier, J. and Vale, W., Intraventricular corticotropin-releasing factor enhances behavioral effects of novelty, *Life Sci.*, 31 (1982) 363-367.
 - 26 Britton, D.R., Hoffman, D.K., Lederis, K. and Rivier, J., A comparison of the behavioral effects of CRF, sauvagine and urotensin I, *Brain Res.*, 304 (1984) 201-205.
 - 27 Britton, D.R., Varela, M., Garcia, A. and Rosenthal, M., Dexamethasone suppresses pituitary-adrenal but not behavioral effects of centrally administered CRF, *Life Sci.*, 38 (1986) 211-216.
 - 28 Britton, D.R. and Indyk, E., Effects of ganglionic blocking agents on behavioral responses to centrally administered CRF, *Brain Res.*, 478 (1989) 205-210.
 - 29 Britton, K.T., Lyon, M., Vale, W. and Koob, G.F., Stress-induced secretion of corticotropin-releasing factor immunoreactivity in rat cerebrospinal fluid, *Soc. Neurosci. Abstr.*, 10 (1984) 94.
 - 30 Britton, K.T., Morgan, J., Rivier, J., Vale, W. and Koob, G.F., Chlordiazepoxide attenuates response suppression induced by corticotropin-releasing factor in the conflict test, *Psychopharmacology*, 86 (1985) 170-174.
 - 31 Britton, K.T., Lee, G., Vale, W., Rivier, J. and Koob, G.F., Corticotropin releasing factor (CRF) receptor antagonist blocks activating and 'anxiogenic' actions of CRF in the rat, *Brain Res.*, 369 (1986) 303-306.
 - 32 Britton, K.T., Lee, G., Dana, R., Risch, S.C. and Koob, G.F., Activating and 'anxiogenic' effects of corticotropin releasing factor are not inhibited by blockade of the pituitary-adrenal system with dexamethasone, *Life Sci.*, 39 (1986) 1281-1286.
 - 33 Britton, K.T., Lee, G. and Koob, G.F., Corticotropin releasing factor and amphetamine exaggerate partial agonist properties of benzodiazepine antagonist Ro 15-1788 in the conflict test, *Psychopharmacology*, 94 (1988) 306-311.
 - 34 Brown, M., Corticotropin releasing factor: central nervous system sites of action, *Brain Res.*, 399 (1986) 10-14.
 - 35 Brown, M.R. and Fisher, L.A., Central nervous system effects of corticotropin releasing factor in the dog, *Brain Res.*, 280 (1983) 75-79.
 - 36 Brown, M.R. and Fisher, L.A., Corticotropin-releasing factor: effects on the autonomic nervous system and visceral systems, *Fed. Proc.*, 44 (1985) 243-248.
 - 37 Brown, M.R., Fisher, L.A., Rivier, J., Spiess, J., Rivier, C. and Vale, W., Corticotropin-releasing factor: effects on the sympathetic nervous system and oxygen consumption, *Life Sci.*, 30 (1982) 207-210.
 - 38 Brown, M.R., Fisher, L.A., Spiess, J., Rivier, C., Rivier, J. and Vale, W., Corticotropin-releasing factor: actions on the sympathetic nervous system and metabolism, *Endocrinology*, 111 (1982) 928-931.
 - 39 Brown, M.R., Fisher, L.A., Webb, V., Vale, W.W. and Rivier, J.E., Corticotropin-releasing factor: a physiologic regulator of adrenal epinephrine secretion, *Brain Res.*, 328 (1985) 355-357.
 - 40 Brown, M.R., Gray, T.S. and Fisher, L.A., Corticotropin-releasing factor receptor antagonist: effects on the autonomic nervous system and cardiovascular function, *Regulat. Pept.*, 16 (1986) 321-329.
 - 41 Buéno, L. and Gué, M., Evidence for the involvement of corticotropin-releasing factor in the gastrointestinal disturbances induced by acoustic and cold stress in mice, *Brain Res.*, 441 (1988) 1-4.
 - 42 Buéno, L., Fargeas, M.J., Gué, M., Peeters, T.L., Bormans, V. and Fioramonti, J., Effects of corticotropin-releasing factor on plasma motilin and somatostatin levels and gastrointestinal motility in dogs, *Gastroenterology*, 91 (1986) 884-889.
 - 43 Busbridge, N.J., Dascombe, M.J., Tilders, F.J.H., Van Oers, J.W.A.M., Linton, E.A. and Rothwell, N.J., Central activation of thermogenesis and fever by interleukin-1 β and interleukin-1 α involves different mechanisms, *Biochem. Biophys. Res. Commun.*, 162 (1989) 591-596.
 - 44 Butler, P.D., Weiss, J.M., Stout, J.C. and Nemeroff, C.B., Corticotropin-releasing factor produces fear-enhancing and behavioral activating effects following infusion into the locus coeruleus, *J. Neurosci.*, 10 (1990) 176-183.
 - 45 Calogero A.E., Gallucci, W.T., Gold, P.W. and Chrousos,

- G.P., Multiple feedback regulatory loops upon rat hypothalamic corticotropin-releasing hormone secretion, potential clinical implications, *J. Clin. Invest.*, 82 (1988) 767-774.
- 46 Cha, C.I. and Foote, S.L., Corticotropin-releasing factor in olivocerebellar climbing-fiber system of monkey (*Saimiri sciureus* and *Macaca fascicularis*): parasagittal and regional organization visualized by immunohistochemistry, *J. Neurosci.*, 8 (1988) 4121-4137.
 - 47 Chappell, P.B., Smith, M.A., Kilts, C.D., Bissette, G., Ritchie, J., Anderson, C. and Nemeroff, C.B., Alterations in corticotropin-releasing factor-like immunoreactivity in discrete rat brain regions after acute and chronic stress, *J. Neurosci.*, 6 (1986) 2908-2914.
 - 48 Chen, F.M., Bilezikjian, L.M., Perrin, M.H., Rivier, J. and Vale, W., Corticotropin releasing factor receptor-mediated stimulation of adenylate cyclase activity in the rat brain, *Brain Res.*, 381 (1986) 49-57.
 - 49 Colbern, D.L., Isaacson, R.L., Green, E.J. and Gispén, W.H., Repeated intraventricular injections of ACTH₁₋₂₄: the effects of home or novel environments on excessive grooming, *Behav. Biol.*, 23 (1978) 381-387.
 - 50 Cole, B.J. and Koob, G.F., Propranolol antagonizes the enhanced conditioned fear produced by corticotropin-releasing factor, *J. Pharmacol. Exp. Ther.*, 247 (1988) 902-910.
 - 51 Cole, B.J. and Koob, G.F., Low doses of corticotropin-releasing factor potentiate amphetamine-induced stereotyped behavior, *Psychopharmacology*, 99 (1989) 27-33.
 - 52 Cole, B.J., Britton, K.T. and Koob, G.F., Central administration of α -helical corticotropin-releasing factor attenuates the acquisition of a conditioned emotional response, *Soc. Neurosci. Abstr.*, 13 (1987) 427.
 - 53 Conaglen, J.V., Donald, R.A., Espiner, E.A., Livesey, J.H. and Nicholls, M.G., Effect of naloxone on the hormone response to CRF in normal man, *Endocrine Res.*, 11 (1985) 39-44.
 - 54 Conte-Devolx, B., Rey, M., Boudouresque, F., Giraud, P., Castanas, E., Millet, Y., Codaccioni, J.L. and Oliver, C., Effect of 41-CRF antiserum on the secretion of ACTH, β -endorphin and α -MSH in the rat, *Peptides*, 4 (1983) 301-304.
 - 55 Crawley, J.N., Olschowka, J.A., Diz, D.I. and Jacobowitz, D.M., Behavioral investigation of the coexistence of substance P, corticotropin releasing factor and acetylcholinesterase in lateral dorsal tegmental neurons projecting to the medial frontal cortex of the rat, *Peptides*, 6 (1985) 891-901.
 - 56 Cummings, S., Sharp, B. and Elde, R., Corticotropin-releasing factor in cerebellar afferent systems: a combined immunohistochemistry and retrograde transport study, *J. Neurosci.*, 8 (1988) 543-554.
 - 57 Cunningham, E.T. and Sawchenko, P.E., Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus, *J. Comp. Neurol.*, 274 (1988) 60-76.
 - 58 Dascombe, M.J., Rothwell, N.J., Sagay, B.O. and Stock, M.J., Pyrogenic and thermogenic effects of interleukin- β in the rat, *Am. J. Physiol.*, 256 (1989) E7-11.
 - 59 Davis, M., Cedarbaum, J.M., Aghajanian, G.K. and Gendelman, D.S., Effects of clonidine on habituation and sensitization of acoustic startle in normal, decerebrate and locus coeruleus lesioned rats, *Psychopharmacology (Berlin)*, 51 (1977) 243-253.
 - 60 De Souza, E.B. and Battaglia, G., Increased corticotropin-releasing factor receptors in rat cerebral cortex following chronic atropine treatment, *Brain Res.*, 397 (1986) 401-404.
 - 61 De Souza, E.B. and Van Loon, G.R., Corticotropin releasing factor increases the adrenocortical responsiveness to adrenocorticotropin, *Experientia*, 40 (1984) 1004-1006.
 - 62 De Souza, E.B., Perrin, M.H., Insel, T.R., Rivier, J., Vale, W.W. and Kuhar, M.J., Corticotropin-releasing factor receptors in rat forebrain: autoradiographic identification, *Science*, 224 (1984) 1449-1451.
 - 63 De Souza, E.B., Insel, T.R., Perrin, M.H., Rivier, J., Vale, W.W. and Kuhar, M.J., Corticotropin-releasing factor receptors are widely distributed within the rat central nervous system: an autoradiographic study, *J. Neurosci.*, 5 (1985) 3189-3203.
 - 64 De Souza, E.B., Whitehouse, P.J., Kuhar, M.J., Price, D.L. and Vale, W.W., Reciprocal changes in corticotropin-releasing factor (CRF)-like immunoreactivity and CRF receptors in cerebral cortex of Alzheimer's disease, *Nature*, 319 (1986) 593-595.
 - 65 Deutch, A.Y., Bean, A.J., Bissette, G., Nemeroff, C.B., Robbins, R.J. and Roth, R.H., Stress-induced alterations in neurotensin, somatostatin and corticotropin-releasing factor in mesolimbic dopamine system regions, *Brain Res.*, 417 (1987) 350-354.
 - 66 Druge, G., Raedler, A., Greten, H. and Lenz, H.J., Pathways mediating CRF-induced inhibition of gastric acid secretion in rats, *Am. J. Physiol.*, 256 (1989) 6214-6219.
 - 67 Dunn, A.J., Effects of ACTH, β -lipotropin and related peptides on the central nervous system. In C.B. Nemeroff and A.J. Dunn (Eds.), *Peptides, Hormones and Behavior: Molecular and Behavioral Neuroendocrinology*, Spectrum, New York, 1984, pp. 273-348.
 - 68 Dunn, A.J., Stress-related changes in cerebral catecholamine and indoleamine metabolism: lack of effect of adrenalectomy and corticosterone, *J. Neurochem.*, 51 (1988) 406-412.
 - 69 Dunn, A.J. and Berridge, C.W., Corticotropin-releasing factor administration elicits a stress-like activation of cerebral catecholaminergic systems, *Pharmacol. Biochem. Behav.*, 27 (1987) 685-691.
 - 70 Dunn, A.J. and File, S.E., Corticotropin-releasing factor has an anxiogenic action in the social interaction test, *Horm. Behav.*, 21 (1987) 193-202.
 - 71 Dunn, A.J. and Hurd, R.W., ACTH acts via anterior third ventricle site to elicit grooming behavior, *Peptides*, 7 (1986) 651-657.
 - 72 Dunn, A.J. and Kramarcy, N.R., Neurochemical responses in stress: relationships between the hypothalamic-pituitary-adrenal and acetylcholine systems. In L.L. Iversen, S.D. Iversen and S.H. Snyder (Eds.), *Handbook of Psychopharmacology, Vol. 18*, Plenum, New York, 1984, pp. 455-515.
 - 73 Dunn, A.J., Guild, A.L., Kramarcy, N.R. and Ware, M.D., Benzodiazepines decrease grooming in response to novelty but not ACTH or β -endorphin, *Pharmacol. Biochem. Behav.*, 15 (1981) 605-608.
 - 74 Dunn, A.J., Berridge, C.W., Lai, Y.I. and Yachabach, T.L., CRF-induced excessive grooming behavior in rats and mice, *Peptides*, 8 (1987) 841-844.
 - 75 Eaves, M., Thatcher-Britton, K., Rivier, J., Vale, W. and Koob, G.F., Effects of corticotropin releasing factor on locomotor activity in hypophysectomized rats, *Peptides*, 6 (1985) 923-926.
 - 76 Eberly, L.B., Dudley, C.A. and Moss, R.L., Ionophoretic mapping of corticotropin-releasing factor (CRF) sensitive neurons in the rat forebrain, *Peptides*, 4 (1983) 837-841.
 - 77 Ehlers, C.L., Henriksen, S.J., Wang, M., Rivier, J., Vale, W. and Bloom, F.E., Corticotropin releasing factor produces increases in brain excitability and convulsive seizures in rats, *Brain Res.*, 278 (1983) 332-336.
 - 78 Ehlers, C.L., Reed, T.K. and Henriksen, S.J., Effects of corticotropin-releasing factor and growth hormone-releasing factor on sleep and activity in rats, *Neuroendocrinology*, 42 (1986) 467-474.
 - 79 Ehlers, C.L., Chaplin, R.I. and Koob, G.F., Antidepressants modulate the CNS effects of corticotropin releasing factor in rats, *Med. Sci. Res.*, 15 (1987) 719-720.
 - 80 Emeric-Sauval, E., Corticotropin-releasing factor (CRF) - a review, *Psychoneuroendocrinology*, 11 (1986) 277-294.
 - 81 File, S.E., The contribution of behavioural studies to the neuropharmacology of anxiety, *Neuropharmacology*, 26 (1987)

- 877-886.
- 82 File, S.E., Johnston, A.L. and Baldwin, H.A., Anxiolytic and anxiogenic drugs: changes in behaviour and endocrine responses, *Stress Med.*, 4 (1988) 221-230.
- 83 Fisher, L.A., Central autonomic modulation of cardiac baroreflex by corticotropin-releasing factor, *Am. J. Physiol.*, 256 (1989) H949-H955.
- 84 Fisher, L.A., Rivier, J., Rivier, C., Spiess, J., Vale, W. and Brown, M.R., Corticotropin-releasing factor (CRF): central effects on mean arterial pressure and heart rate in rats, *Endocrinology*, 110 (1982) 2222-2224.
- 85 Fisher, L.A., Jessen, G. and Brown, M.R., Corticotropin-releasing factor (CRF): mechanism to elevate mean arterial pressure and heart rate, *Regulat. Pept.*, 5 (1983) 153-161.
- 86 Foote, S.L. and Cha, C.I., Distribution of corticotropin-releasing-factor-like immunoreactivity in brain-stem of two monkey species (*Saimiri sciureus* and *Macaca fascicularis*): an immunohistochemical study, *J. Comp. Neurol.*, 276 (1988) 239-264.
- 87 Gambacciani, M., Yen, S.S.C. and Rasmussen, D.D., GnRH release from the mediobasal hypothalamus: in vitro inhibition by corticotropin-releasing factor, *Neuroendocrinology*, 43 (1986) 533-536.
- 88 Garrick, T., Veisich, A., Sierra, A., Weiner, H. and Taché, Y., Corticotropin-releasing factor acts centrally to suppress stimulated gastric contractility in the rat, *Regulat. Pept.*, 21 (1988) 173-181.
- 89 Giguère, V., Labrie, F., Côté, J., Coy, D.H., Sueiras-Diaz, J. and Schally, A.V., Stimulation of cyclic AMP accumulation and corticotropin release by synthetic ovine corticotropin-releasing factor in rat anterior pituitary cells: site of glucocorticoid action, *Proc. Natl. Acad. Sci. U.S.A.*, 79 (1982) 3466-3469.
- 90 Gindoff, P.R. and Ferin, M., Endogenous opioid peptides modulate the effect of corticotropin-releasing factor on gonadotropin release in the primate, *Endocrinology*, 121 (1987) 837-842.
- 91 Goeders, N.R., Bienvenu, O.J. and De Souza, E.B., Chronic cocaine administration alters corticotropin-releasing factor receptors in the rat brain, *Brain Res.*, in press.
- 92 Gold, P.W., Chrousos, G., Kellner, C., Post, R., Roy, A., Augerinos, P., Schulte, H., Oldfield, E. and Loriaux, D.L., Psychiatric implications of basic and clinical studies with corticotropin-releasing factor, *Am. J. Psychiat.*, 141 (1984) 619-627.
- 93 Gold, P.W. and Chrousos, G.P., Clinical studies with corticotropin releasing factor: implications for the diagnosis and pathophysiology of depression, Cushing's disease, and adrenal insufficiency, *Psychoneuroendocrinology*, 10 (1985) 401-419.
- 94 Gosnell, B.A., Morley, J.E. and Levine, A.S., A comparison of the effects of corticotropin releasing factor and sauvagine on food intake, *Pharmacol. Biochem. Behav.*, 19 (1983) 771-775.
- 95 Goto, Y. and Taché, Y., Gastric erosions induced by intracisternal thyrotropin-releasing hormone (TRH) in rats, *Peptides*, 6 (1985) 153-156.
- 96 Grigoriadis, D.E., Pearsall, D. and De Souza, E.B., Effects of chronic antidepressant and benzodiazepine treatment on corticotropin-releasing-factor receptors in rat brain and pituitary, *Neuropsychopharmacology*, 2 (1989) 53-60.
- 97 Grosskreutz, C.L. and Brody, M.J., Regional hemodynamic responses to central administration of corticotropin-releasing factor (CRF), *Brain Res.*, 442 (1988) 363-367.
- 98 Gué, M. and Buéno, L., Involvement of CNS corticotropin-releasing factor in the genesis of stress-induced gastric motor alterations. In Singer and Goebell (Eds.), *Nerves and the Gastrointestinal Tract*, 1989, pp. 217-225.
- 99 Gué, M., Fioramonti, J., Frexinos, J., Alvinerie, M. and Buéno, L., Influence of acoustic stress by noise on gastrointestinal motility in dogs, *Dig. Dis. Sci.*, 32 (1987) 1411-1417.
- 100 Gué, M., Fioramonti, J. and Buéno, L., Comparative influences of acoustic and cold stress on gastrointestinal transit in mice, *Am. J. Physiol.*, 253 (1987) G124-128.
- 101 Guillemin, R. and Rosenberg, B., Humoral hypothalamic control of anterior pituitary: a study with combined tissue cultures, *Endocrinology*, 57 (1955) 599-607.
- 102 Guinon, M.W. and Taché, Y., Intrahypothalamic microinfusion of corticotropin-releasing factor inhibits gastric acid secretion but increases secretion volume in rats, *Brain Res.*, 411 (1987) 156-161.
- 103 Guinon, M.W., Kauffman, G.L. and Taché, Y., Intrahypothalamic corticotropin-releasing factor elevates gastric bicarbonate and inhibits stress ulcers in rats, *Am. J. Physiol.*, 258 (1990) G152-157.
- 104 Hargreaves, K.M., Mueller, G.P., Dubner, R., Goldstein, D. and Dionne, R.A., Corticotropin-releasing factor (CRF) produces analgesia in humans and rats, *Brain Res.*, 422 (1987) 154-157.
- 105 Hargreaves, K.M., Dubner, R. and Costello, A.H., Corticotropin releasing factor (CRF) has a peripheral site of action for antinociception, *Eur. J. Pharmacol.*, 170 (1989) 275-279.
- 106 Hargreaves, K.M., Flores, C.M., Dionne, R.A. and Mueller, G.P., The role of pituitary β -endorphin in mediating corticotropin-releasing factor-induced antinociception, *Am. J. Physiol.*, 258 (1990) E235-242.
- 107 Harris, G.W., Neural control of the pituitary gland, *Physiol. Rev.*, 28 (1948) 139-179.
- 108 Hauger, R.L., Millan, M.A., Catt, K.J. and Aguilera, G., Differential regulation of brain and pituitary corticotropin-releasing factor receptors by corticosterone, *Endocrinology*, 120 (1987) 1527-1533.
- 109 Hauger, R.L., Millan, M.A., Lorang, M., Harwood, J.P. and Aguilera, G., Corticotropin-releasing factor receptors and pituitary adrenal responses during immobilization stress, *Endocrinology*, 123 (1988) 396-405.
- 110 Hoffman, W.E. and Phillips, M.I., Regional study of cerebral ventricle sensitive sites to angiotensin II, *Brain Res.*, 110 (1976) 313-330.
- 111 Holsboer, F., Gerken, A., Stalla, G.K. and Müller, O.A., Blunted aldosterone and ACTH release after human CRH administration in depressed patients, *Am. J. Psychiatry*, 144 (1987) 229-231.
- 112 Honour, L.C. and White, M.H., Pre- and postnatally administered ACTH, Organon 2766 and CRF facilitate or inhibit active avoidance task performance in young adult mice, *Peptides*, 9 (1988) 745-750.
- 113 Hornby, P.J. and Piekut, D.T., Opiocortin and catecholamine input to CRF-immunoreactive neurons in rat forebrain, *Peptides*, 10 (1989) 1139-1146.
- 114 Imaki, T., Shibasaki, T., Masuda, A., Demura, H., Shizume, K. and Ling, N., Effects of adrenergic blockers on corticotropin-releasing factor-induced behavioral changes in rats, *Regulat. Pept.*, 19 (1987) 243-252.
- 115 Imaki, T., Shibasaki, T., Masuda, A., Imaki, J., Demura, H., Shizume, K. and Ling, N., Corticotropin-releasing factor reverses the effect of pentobarbital through a β -noradrenergic mechanism in rats, *Life Sci.*, 43 (1988) 813-820.
- 116 Insel, T.R., Aloï, J.A., Goldstein, D., Wood, J.H. and Jimerson, D.C., Plasma cortisol and catecholamine responses to intracerebroventricular administration of CRF to rhesus monkeys, *Life Sci.*, 34 (1984) 1873-1878.
- 117 Irwin, M.R., Vale, W. and Britton, K.T., Central corticotropin-releasing factor suppresses natural killer cytotoxicity, *Brain Behav. Immun.*, 1 (1987) 81-87.
- 118 Irwin, M., Hauger, R.L., Brown, M. and Britton, K.T., CRF activates autonomic nervous system and reduces natural killer cytotoxicity, *Am. J. Physiol.*, 255 (1988) R744-R747.
- 119 Irwin, M., Vale, W. and Rivier, C., Central corticotropin-releasing factor mediates the suppressive effect of stress on natural killer cytotoxicity, *Endocrinology*, 126 (1990) 2837-2844.
- 120 Kalin, N.H., Shelton, S.E., Kraemer, G.W. and McKinney,

- W.T., Associated endocrine, physiological and behavioral changes in rhesus-monkeys after intravenous corticotropin-releasing factor administration, *Peptides*, 4 (1983) 211-215.
- 121 Kalin, N.H., Shelton, S.E., Kraemer, G.W. and McKinney, W.T., Corticotropin-releasing factor administered intraventricularly to rhesus monkeys, *Peptides*, 4 (1983) 217-220.
 - 122 Kalin, N.H., Sherman, J.E. and Takahashi, L.K., Antagonism of endogenous CRH systems attenuates stress-induced freezing behavior in rats, *Brain Res.*, 457 (1988) 130-135.
 - 123 Kalin, N.H., Shelton, S.E. and Barksdale, C.M., Behavioral and physiological effects of CRH administered to infant primates undergoing maternal separation, *Neuropsychopharmacology*, 2 (1989) 97-104.
 - 124 Kalin, N.H. and Takahashi, L.K., Fear-motivated behavior induced by prior shock experience is mediated by corticotropin-releasing hormone systems, *Brain Res.*, 509 (1990) 80-84.
 - 125 Kalivas, P.W., Duffy, P. and Laimer, L.G., Neurochemical and behavioral effects of corticotropin-releasing factor in the ventral tegmental area of the rat, *J. Pharmacol. Exp. Ther.*, 242 (1987) 757-764.
 - 126 Karlsson, A. and Ahren, B., Effects of corticotropin-releasing hormone on insulin and glucagon secretion in mice, *Acta Endocrinol.*, 117 (1988) 87-92.
 - 127 Katakami, H., Arimura, A. and Frohman, L.A., Involvement of hypothalamic somatostatin in the suppression of growth hormone secretion by central corticotropin-releasing factor in conscious male rats, *Neuroendocrinology*, 41 (1985) 390-393.
 - 128 Kavelaars, A., Ballieux, R.E. and Heijnen, C.J., The role of IL-1 in the corticotropin-releasing factor and arginine-vasopressin-induced secretion of immunoreactive β -endorphin by human peripheral blood mononuclear cells, *J. Immunol.*, 142 (1989) 2338-2342.
 - 129 Kavelaars, A., Berkenbosch, F., Croiset, G., Ballieux, R.E. and Heijnen, C.J., Induction of β -endorphin secretion by lymphocytes after subcutaneous administration of corticotropin-releasing factor, *Endocrinology*, 126 (1990) 759-764.
 - 130 Kiang, J.G. and Wei, E.T., CRF-evoked bradycardia in urethane-anesthetized rats is blocked by naloxone, *Peptides*, 6 (1985) 409-413.
 - 131 Konturek, S.J., Bilski, J., Pawlik, W., Thor, P., Czarnobilski, K., Szoke, B. and Schally, A.V., Gastrointestinal secretory, motor and circulatory effects of corticotropin-releasing factor (CRF), *Life Sci.*, 37 (1985) 1231-1240.
 - 132 Koob, G.F., Swerdlow, N., Seeligson, M., Eaves, M., Sutton, R., Rivier, J. and Vale, W., Effects of α -flupenthixol and naloxone on CRF-induced locomotor activation, *Neuroendocrinology*, 39 (1984) 459-464.
 - 133 Koob, G.F. and Bloom, F.E., Corticotropin-releasing factor and behavior, *Fed. Proc.*, 44 (1985) 259-263.
 - 134 Krahn, D.D., Gosnell, B.A., Grace, M. and Levine, A.S., CRF antagonist partially reverses CRF- and stress-induced effects on feeding, *Brain Res. Bull.*, 17 (1986) 285-289.
 - 135 Krahn, D.D., Wright, B., Billington, C.J. and Levine, A.S., Exogenous corticotropin-releasing factor inhibits stress-induced gastric ulceration, *Soc. Neurosci. Abstr.*, 12 (1986) 1063.
 - 136 Krahn, D.D., Gosnell, B.A., Levine, A.S. and Morley, J.E., Behavioral effects of corticotropin-releasing factor: localization and characterization of central effects, *Brain Res.*, 443 (1988) 63-69.
 - 137 Kurosawa, M., Sato, A., Swenson, R.S. and Takahashi, Y., Sympatho-adrenal medullary functions in response to intracerebroventricularly injected corticotropin-releasing factor in anesthetized rats, *Brain Res.*, 367 (1986) 250-257.
 - 138 Lee, E.H.Y. and Sung, Y.J., Differential influences of corticotropin-releasing factor on memory retention of aversive learning and appetitive learning in rats, *Behav. Neural Biol.*, 52 (1989) 285-294.
 - 139 Lee, E.H.Y. and Tsai, M.J., The hippocampus and amygdala mediate the locomotor stimulating effects of corticotropin-releasing factor in mice, *Behav. Neural Biol.*, 51 (1989) 412-423.
 - 140 Lee, E.H.Y., Tang, Y.P. and Chai, C.Y., Stress and corticotropin-releasing factor potentiate center region activity of mice in an open field, *Psychopharmacology*, 93 (1987) 320-324.
 - 141 Lenz, H.J., Hester, S.E. and Brown, M.R., Corticotropin-releasing factor. Mechanisms to inhibit gastric acid secretion in conscious dogs, *J. Clin. Invest.*, 75 (1985) 889-895.
 - 142 Lenz, H.J., Raedler, A., Greten, H. and Brown, M.R., CRF initiates biological actions within the brain that are observed in response to stress, *Am. J. Physiol.*, 252 (1987) R34-R39.
 - 143 Lenz, H.J., Burlage, M., Raedler, A. and Greten, H., Central nervous system effects of corticotropin-releasing factor on gastrointestinal transit in the rat, *Gastroenterology*, 94 (1988) 598-602.
 - 144 Lenz, H.J., Raedler, A., Greten, H., Vale, W.W. and Rivier, J.E., Stress-induced gastrointestinal secretory and motor responses in rats are mediated by endogenous corticotropin-releasing factor, *Gastroenterology*, 95 (1988) 1510-1517.
 - 145 Levine, A.S., Rogers, B., Kneip, J., Grace, M. and Morley, J.E., Effect of centrally administered corticotropin releasing factor (CRF) on multiple feeding paradigms, *Neuropharmacology*, 22 (1983) 337-339.
 - 146 Lewis, R.E. and Phillips, M.I., Localization of the central pressor action of bradykinin to the cerebral third ventricle, *Am. J. Physiol.*, 247 (1984) R63-R68.
 - 147 Liang, K.C. and Lee, E.H.Y., Intra-amygdala injections of corticotropin releasing factor facilitate inhibitory avoidance learning and reduce exploratory behavior in rats, *Psychopharmacology*, 96 (1988) 232-236.
 - 148 Liang, K.C., Miserendino, M.J.D., Melia, K.R. and Davis, M., Corticotropin-releasing factor enhances the acoustic startle reflex: involvement of the amygdala and the spinal cord, *Soc. Neurosci. Abstr.*, 15 (1989) 1069.
 - 149 Liebeskind, J.C., Dismukes, R.K., Barker, J.L., Berger, P.A., Creese, I., Dunn, A.J., Segal, D.S., Stein, L. and Vale, W.W., Peptides and behavior: a critical analysis of research strategies, *Neurosci. Res. Prog. Bull.*, 16 (1978) 490-635.
 - 150 Linton, E.A., Tilders, F.J.H., Hodgkinson, S., Berkenbosch, F., Verme, I. and Lowry, P.J., Stress-induced secretion of adrenocorticotropin in rats is inhibited by administration of antisera to ovine corticotropin-releasing factor and vasopressin, *Endocrinology*, 116 (1985) 966-970.
 - 151 Liposits, Zs. and Paull, W.K., Association of dopaminergic fibers with corticotropin releasing hormone (CRH)-synthesizing neurons in the paraventricular nucleus of the rat hypothalamus, *Histochemistry*, 93 (1989) 119-127.
 - 152 Martin, J.B. and Reichlin, S., *Clinical Neuroendocrinology*, 2nd edn., F.A. Davis, Philadelphia, PA, 1987, 759 pp.
 - 153 Marrosu, F., Mereu, G., Fratta, W., Carcangiu, P., Camarri, F. and Gessa, G.L., Different epileptogenic activities of murine and ovine corticotropin-releasing factor, *Brain Res.*, 408 (1987) 394-398.
 - 154 Mason, J.W., Organization of the multiple endocrine responses to avoidance in the monkey, *Psychosomat. Med.*, 30 (1968) 774-790.
 - 155 Mason, J.W., A re-evaluation of the concept of 'non-specificity' in stress theory, *J. Psychiatr. Res.*, 8 (1971) 323-333.
 - 156 Matsuzaki, I., Takamatsu, Y. and Moroji, T., The effects of intracerebroventricularly injected corticotropin-releasing factor (CRF) on the central nervous system: behavioral and biochemical studies, *Neuropeptides*, 13 (1989) 147-155.
 - 157 McGillis, J.P., Park, A., Rubin-Fleeter, P., Turck, C., Dallman, M.F. and Payan, D.G., Stimulation of rat β -lymphocyte proliferation by corticotropin-releasing factor, *J. Neurosci. Res.*, 23 (1989) 346-352.
 - 158 Merchenthaler, I., Vigh, S., Schally, A.V., Stumpf, W.E. and Arimura, A., Immunocytochemical localization of corticotropin releasing factor (CRF)-like immunoreactivity in the thalamus of the rat, *Brain Res.*, 323 (1984) 119-122.
 - 159 Miskowiak, B., Janicki, A., Jakubowski, A. and Limanowski,

- A., Reproductive functions in the adult male rats after prolonged intraventricular administration of corticotropin-releasing factor (CRF), *Exp. Clin. Endocrinol.*, 88 (1986) 25-30.
- 160 Mitsuma, T., Nogimori, T. and Hirooka, Y., Effects of growth hormone-releasing hormone and corticotropin-releasing hormone on the release of thyrotropin-releasing hormone from the rat hypothalamus in vitro, *Exp. Clin. Endocrinol.* 90 (1987) 365-368.
 - 161 Moga, M.M. and Gray, T.S., Evidence for corticotropin-releasing factor, neurotensin and somatostatin in the neural pathway from the central nucleus of the amygdala to the parabrachial nucleus, *J. Comp. Neurol.*, 241 (1985) 275-284.
 - 162 Morel, G., Enjalbert, A., Proulx, L., Pelletier, G., Barden, N., Grossard, F. and Dubois, P.M., Effect of corticotropin-releasing factor on the release and synthesis of prolactin, *Neuroendocrinology*, 49 (1989) 669-675.
 - 163 Morley, J.E. and Levine, A.S., Corticotropin releasing factor, grooming and ingestive behavior, *Life Sci.*, 31 (1982) 1459-1464.
 - 164 Nakane, T., Audhya, T., Kanie, N. and Hollander, C.S., Evidence for a role of endogenous corticotropin-releasing factor in cold, ether, immobilization and traumatic stress, *Proc. Natl. Acad. Sci. U.S.A.*, 82 (1985) 1247-1251.
 - 165 Nakane, T., Kanie, N., Audhya, T. and Hollander, C.S., The effects of centrally administered neuropeptides on the development of gastric lesions in the rat, *Life Sci.*, 36 (1985) 1197-1203.
 - 166 Nakane, T., Audhya, T., Hollander, C.S., Schlesinger, D.H., Kardos, P., Brown, C. and Passarelli, J., Corticotropin-releasing factor in extra-hypothalamic brain of the mouse: demonstration by immunoassay and immunoneutralization of bioassayable activity, *J. Endocrinol.*, 111 (1986) 143-149.
 - 167 Nemeroff, C.B., Widerlöv, E., Bissette, G., Walléus, Karlsson, I., Eklund, K., Kilts, C.D., Loosen, P.T. and Vale, W., Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients, *Science*, 226 (1984) 1342-1344.
 - 168 Nemeroff, C.B., The role of corticotropin-releasing factor in the pathogenesis of major depression, *Pharmacopsychiatry*, 21 (1988) 76-82.
 - 169 Nemeroff, C.B., Owens, M.J., Bissette, G., Andorn, A.C. and Stanley, M., Reduced corticotropin releasing factor binding sites in the frontal cortex of suicide victims, *Arch. Gen. Psychiat.* 45 (1988) 577-579.
 - 170 Nikolarakis, K.E., Almeida, O.F.X. and Herz, A., Corticotropin-releasing factor (CRF) inhibits gonadotropin-releasing hormone (GnRH) release from superfused rat hypothalamus in vitro, *Brain Res.*, 377 (1986) 388-390.
 - 171 Nikolarakis, K.E., Almeida, O.F.X. and Herz, A., Hypothalamic opioid receptors mediate the inhibitory actions of corticotropin-releasing hormone on luteinizing hormone release: further evidence from a morphine-tolerant animal model, *Brain Res.*, 450 (1988) 360-363.
 - 172 Olanas, M.C. and Onali, P., Corticotropin-releasing factor activates tyrosine hydroxylase in rat and mouse striatal homogenates, *Eur. J. Pharmacol.*, 150 (1988) 389-392.
 - 173 Olanas, M.C. and Onali, P., Stimulation of synaptosomal dopamine synthesis by corticotropin-releasing factor in rat striatum: role of Ca^{2+} -dependent mechanisms, *Eur. J. Pharmacol.*, 166 (1989) 165-174.
 - 174 Olster, D.H. and Ferin, M., Corticotropin-releasing hormone inhibits gonadotropin secretion in the ovariectomized rhesus monkey, *J. Clin. Endocrinol. Metab.*, 65 (1987) 262-267.
 - 175 Ono, N., Lumpkin, M.D., Samson, W.K., McDonald, J.K. and McCann, S.M., Intrahypothalamic action of corticotropin-releasing factor (CRF) to inhibit growth hormone and LH release in the rat, *Life Sci.*, 35 (1984) 1117-1123.
 - 176 Ono, N., De Castro, J.C.B. and McCann, S.M., Ultrashort-loop positive feedback of corticotropin (ACTH)-releasing factor to enhance ACTH in stress, *Proc. Natl. Acad. Sci. U.S.A.*, 82 (1985) 3528-3531.
 - 177 Ono, N., Samson, W.K., McDonald, J.K., Lumpkin, M.D., Bedran de Castro, J.C. and McCann, S.M., Effects of intravenous and intraventricular injection of antisera directed against corticotropin-releasing factor on the secretion of anterior pituitary hormones, *Proc. Natl. Acad. Sci. U.S.A.*, 82 (1985) 7787-7790.
 - 178 Owens, M.J., Bissette, G. and Nemeroff, C.B., Acute effects of alprazolam and adinazolam on the concentrations of corticotropin-releasing factor in rat brain, *Synapse*, 4 (1989) 196-202.
 - 179 Pappas, T., Debas, H. and Taché, Y., Corticotropin-releasing factor inhibits gastric emptying in dogs, *Regulat. Pept.*, 11 (1985) 193-199.
 - 180 Pappas, T.N., Welton, M., Debas, H.T., Rivier, J. and Taché, Y., Corticotropin-releasing factor inhibits gastric emptying in dogs: studies on its mechanism of action, *Peptides*, 8 (1987) 1011-1014.
 - 181 Pawlikowski, M., Zelazowski, P., Döhler, K. and Stepien, H., Effects of two neuropeptides, somatoliberin (GRF) and corticoliberin (CRF) on human lymphocyte natural killer activity, *Brain Behav. Immunol.* 2 (1988) 50-56.
 - 182 Petraglia, F., Vale, W. and Rivier, C., Opioids act centrally to modulate stress-induced decrease in luteinizing hormone in the rat, *Endocrinology*, 119 (1986) 2445-2450.
 - 183 Petraglia, F., Sutton, S., Vale, W. and Plotsky, P., Corticotropin-releasing factor decreases plasma luteinizing hormone levels in female rats by inhibiting gonadotropin-releasing hormone release into hypophysial-portal circulation, *Endocrinology*, 120 (1987) 1083-1088.
 - 184 Plotsky, P.M., Facilitation of immunoreactive corticotropin-releasing factor secretion into the hypophysial-portal circulation after activation of catecholaminergic pathways or central norepinephrine injection, *Endocrinology*, 121 (1987) 924-930.
 - 185 Plotsky, P.M., Cunningham, E.T. and Widmaier, E.P., Catecholaminergic modulation of corticotropin-releasing factor and adrenocorticotropin secretion, *Endocr. Rev.*, 10 (1989) 437-458.
 - 186 Powers, R.E., De Souza, E.B., Walker, L.C., Price, D.L., Vale, W.W. and Young, W.S., Corticotropin-releasing factor as a transmitter in the human olivocerebellar pathway, *Brain Res.*, 415 (1987) 347-352.
 - 187 Rivier, C.L. and Plotsky, P.M., Mediation by corticotropin releasing factor (CRF) of adeno-hypophysial hormone secretion, *Annu. Rev. Physiol.*, 48 (1986) 475-494.
 - 188 Rivier, C. and Vale, W., Influence of corticotropin-releasing factor on reproductive functions in the rat, *Endocrinology*, 114 (1984) 914-921.
 - 189 Rivier, C. and Vale, W., Corticotropin-releasing factor (CRF) acts centrally to inhibit growth hormone secretion in the rat, *Endocrinology*, 114 (1984) 2409-2411.
 - 190 Rivier, C. and Vale, W., Effect of the long-term administration of CRF on the pituitary-adrenal and pituitary-gonadal axis in the male rat, *J. Clin. Invest.*, 75 (1985) 689-694.
 - 191 Rivier, C. and Vale, W., Involvement of corticotropin-releasing factor and somatostatin in stress-induced inhibition of growth hormone secretion in the rat, *Endocrinology*, 117 (1985) 2478-2482.
 - 192 Rivier, C. and Vale, W., Neuroendocrine interaction between corticotropin releasing factor and vasopressin on adrenocorticotrophic hormone secretion in the rat. In R.W. Schrier (Ed.), *Vasopressin*, Raven Press, New York, 1985, pp. 181-188.
 - 193 Rivier, C., Rivier, J. and Vale, W., Inhibition of adrenocorticotrophic hormone secretion in the rat by immunoneutralization of corticotropin-releasing factor, *Science*, 218 (1982) 377-379.
 - 194 Rivier, J., Rivier, C. and Vale, W., Synthetic competitive antagonists of corticotropin-releasing factor: effects on ACTH secretion in the rat, *Science*, 224 (1984) 889-891.
 - 195 Rivier, C., Rivier, J. and Vale, W., Stress-induced inhibition of reproductive functions: role of endogenous corticotropin-re-

- leasing factor, *Science*, 231 (1986) 607-609.
- 196 Rock, J.P., Oldenfield, E.H., Schulte, H.M., Gold, P.W., Kornblith, P.L., Loriaux, L. and Chrousos, G.P., Corticotropin releasing factor administered into the ventricular CSF stimulates the pituitary-adrenal axis, *Brain Res.*, 323 (1984) 365-368.
 - 197 Rosenthal, M.J. and Morley, J.E., Corticotropin releasing factor (CRF) and age-related differences in behavior of mice, *Neurobiol. Aging*, 10 (1989) 167-171.
 - 198 Roth, K.A. and Katz, R.J., Stress, behavioral arousal, and open field activity - a reexamination of emotionality in the rat, *Neurosci. Biobehav. Rev.*, 3 (1979) 247-263.
 - 199 Rothwell, N.J., CRF is involved in the pyrogenic and thermogenic effects of interleukin 1 β in the rat, *Am. J. Physiol.*, 256 (1989) E111-E115.
 - 200 Ruckebusch, Y. and Malbert, C.H., Stimulation and inhibition of food intake in sheep by centrally administered hypothalamic releasing factors, *Life Sci.*, 38 (1986) 929-934.
 - 201 Saffran, M. and Schally, A.V., The release of corticotropin by anterior pituitary tissue in vivo, *Can. J. Biochem. Physiol.*, 33 (1955) 408-415.
 - 202 Saffran, M. and Schally, A.V., The status of the corticotropin releasing factor (CRF), *Neuroendocrinology*, 24 (1977) 359-375.
 - 203 Sahgal, A., Wright, C., Edwardson, J.A. and Keith, A.B., Corticotropin releasing factor is more potent than some corticotropin-related peptides in affecting passive avoidance behaviour in rats, *Neurosci. Lett.*, 36 (1983) 81-86.
 - 204 Sakanaka, M., Shibasaki, T. and Lederis, K., Corticotropin releasing factor-like immunoreactivity in the rat brain as revealed by a modified cobalt-glucose oxidase-diaminobenzidine method, *J. Comp. Neurol.*, 260 (1987) 256-298.
 - 205 Sakanaka, M., Magari, S., Shibasaki, T. and Lederis, K., Corticotropin releasing factor-containing afferents to the lateral septum of the rat brain, *J. Comp. Neurol.*, 270 (1988) 404-415.
 - 206 Sakanaka, M., Magari, S., Shibasaki, T. and Inoue, N., Co-localization of corticotropin-releasing factor- and enkephalin-like immunoreactivities in nerve cells of the rat hypothalamus and adjacent areas, *Brain Res.*, 487 (1989) 357-362.
 - 207 Saunders, W.S. and Thornhill, J.A., Pressor, tachycardic and behavioral excitatory responses in conscious rats following i.c.v. administration of ACTH and CRF are blocked by naloxone pretreatment, *Peptides*, 7 (1986) 597-601.
 - 208 Sawchenko, P.E., Evidence for differential regulation of corticotropin-releasing factor and vasopressin immunoreactivities in parvocellular neurosecretory and autonomic-related projections of the paraventricular nucleus, *Brain Res.*, 437 (1987) 253-263.
 - 209 Sawchenko, P.E. and Swanson, L.W., Localization, colocalization and plasticity of corticotropin-releasing factor immunoreactivity in rat brain, *Fed. Proc.*, 44 (1985) 221-227.
 - 210 Sharkey, J., Appel, N.M. and De Souza, E.B., Alterations in local cerebral glucose utilization following central administration of corticotropin-releasing factor in rats, *Synapse*, 4 (1989) 80-87.
 - 211 Sherman, J.E. and Kalin, N.H., I.c.v.-CRH potently affects behavior without altering antinociceptive responding, *Life Sci.*, 39 (1986) 433-441.
 - 212 Sherman, J.E. and Kalin, N.H., I.c.v.-CRH alters stress-induced freezing behavior without affecting pain sensitivity, *Pharmacol. Biochem. Behav.*, 30 (1988) 801-807.
 - 213 Siggins, G.R., Gruol, D., Aldenhoff, J. and Pittman, Q., Electrophysiological actions of corticotropin-releasing factor in the central nervous system, *Fed. Proc.*, 44 (1985) 237-242.
 - 214 Singh, V.B., Corley, K.C., Phan, T.-H. and Boodle-Biber, M.C., Increase in rat cortical and midbrain tryptophan hydroxylase by intracerebroventricular infusion of corticotropin releasing factor, *FASEB J.*, 3 (1989) 2977 Abstr.
 - 215 Singh, V.B., Phan, T.-H. and Boodle-Biber, M.C., Participation of amygdaloid central nucleus (ACE) in CRF induced increases in in vitro tryptophan hydroxylase activity (TrpH), *Soc. Neurosci. Abstr.*, 15 (1989) 215.
 - 216 Singh, V.K., Stimulatory effect of corticotropin-releasing neuropeptide on human lymphocyte proliferation and interleukin-2 receptor expression, *J. Neuroimmunol.*, 23 (1989) 257-262.
 - 217 Singh, V.K. and Fudenberg, H.H., Binding of [¹²⁵I]corticotropin releasing factor to blood immunocytes and its reduction in Alzheimer's disease, *Immunol. Lett.*, 18 (1988) 5-8.
 - 218 Sirinathsinghji, D.J.S., Modulation of lordosis behaviour in the female rat by corticotropin releasing factor, β -endorphin and gonadotropin releasing hormone in the mesencephalic central gray, *Brain Res.*, 336 (1985) 45-55.
 - 219 Sirinathsinghji, D.J.S., Regulation of lordosis behaviour in the female rat by corticotropin-releasing factor, β -endorphin/corticotropin and luteinizing hormone-releasing hormone neuronal systems in the medial preoptic area, *Brain Res.*, 375 (1986) 49-56.
 - 220 Sirinathsinghji, D.J.S., Inhibitory influence of corticotropin releasing factor on components of sexual behaviour in the male rat, *Brain Res.*, 407 (1987) 185-190.
 - 221 Sirinathsinghji, D.J.S. and Heavens, R.P., Stimulation of GABA release from the rat neostriatum and globus pallidus in vivo by corticotropin releasing factor, *Neurosci. Lett.*, 100 (1989) 203-209.
 - 222 Sirinathsinghji, D.J.S., Nikolarakis, K.E. and Herz, A., Corticotropin-releasing factor stimulates the release of methionine-enkephalin and dynorphin from the neostriatum and globus pallidus of the rat: in vitro and in vivo studies, *Brain Res.*, 490 (1989) 276-291.
 - 223 Sirinathsinghji, D.J.S., Rees, L.H., Rivier, J. and Vale, W., Corticotropin-releasing factor is a potent inhibitor of sexual receptivity in the female rat, *Nature*, 305 (1983) 232-235.
 - 224 Smith, M.A., Bissette, G., Slotkin, T.A., Knight, D.L. and Nemeroff, C.B., Release of corticotropin-releasing factor from rat brain regions in vitro, *Endocrinology*, 118 (1986) 1997-2001.
 - 225 Smith, M.A., Kling, M.A., Whitfield, H.J., Brandt, H.A., Demitrack, M.A., Geraciotti, T.D., Chrousos, G.P. and Gold, P.W., Corticotropin-releasing hormone: from endocrinology and psychobiology, *Horm. Res.*, 31 (1989) 66-71.
 - 226 Spadaro, F., Berridge, C.W., Baldwin, H.A. and Dunn, A.J., Corticotropin-releasing factor acts via a third ventricle site to reduce exploratory behavior in rats, *Pharmacol. Biochem. Behav.*, 36 (1990) 305-309.
 - 227 Stephens, R.L., Yang, H., Rivier, J. and Taché, Y., Intracisternal injection of CRF antagonist blocks surgical stress-induced inhibition of gastric secretion in the rat, *Peptides*, 9 (1988) 1067-1070.
 - 228 Stepien, H., Zelazowski, P., Pawlikowski, M. and Dohler, K.D., Corticotropin releasing factor (CRF) suppression of human peripheral blood leukocyte chemotaxis, *Neuroendocrinol. Lett.*, 9 (1987) 225-230.
 - 229 Suda, T., Yajima, F., Tomori, N., Demura, H. and Shizume, K., In vitro study of immunoreactive corticotropin-releasing factor release from the rat hypothalamus, *Life Sci.*, 37 (1985) 1499-1505.
 - 230 Sutton, R.E., Koob, G.F., Le Moal, M., Rivier, J. and Vale, W., Corticotropin releasing factor produces behavioural activation in rats, *Nature*, 297 (1982) 331-333.
 - 231 Swanson, L.W., Sawchenko, P.E., Rivier, J. and Vale, W.W., Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study, *Neuroendocrinology*, 36 (1983) 165-186.
 - 232 Swerdlow, N.R. and Koob, G.F., Separate neural substrates of the locomotor-activating properties of amphetamine, heroin, caffeine and corticotropin releasing factor (CRF) in the rat, *Pharmacol. Biochem. Behav.*, 23 (1985) 303-307.
 - 233 Swerdlow, N.R., Geyer, M.A., Vale, W.W. and Koob, G.F., Corticotropin-releasing factor potentiates acoustic startle in

- rats: blockade by chlordiazepoxide, *Psychopharmacology*, 88 (1986) 147-152.
- 234 Swerdlow, N.R., Britton, K.T. and Koob, G.F., Potentiation of acoustic startle by corticotropin-releasing factor (CRF) and by fear are both reversed by α -helical CRF (9-41), *Neuropsychopharmacology*, 2 (1989) 285-292.
 - 235 Szafarczyk, A., Malaval, F., Laurent, A., Gibaud, R. and Assenmacher, I., Further evidence for a central stimulatory action of catecholamines on adrenocorticotropin release in the rat, *Endocrinology*, 121 (1987) 883-892.
 - 236 Taché, Y., Goto, Y., Gunion, M.W., Vale, W., Rivier, J. and Brown, M., Inhibition of gastric acid secretion in rats by intracerebral injection of corticotropin-releasing factor, *Science*, 222 (1983) 935-937.
 - 237 Taché, Y., Goto, Y., Gunion, M., Rivier, J. and Debas, H., Inhibition of gastric acid secretion in rats and dogs by corticotropin-releasing factor, *Gastroenterology*, 86 (1984) 281-286.
 - 238 Taché, Y., Maeda-Hagiwara, M. and Turkelson, C.M., Central nervous system action of corticotropin-releasing factor to inhibit gastric emptying in rats, *Am. J. Physiol.*, 253 (1987) G241-G245.
 - 239 Taché, Y., Stephens, R.L. and Ishikawa, T., Stress-induced alterations of gastrointestinal function: involvement of brain CRF and TRH. In H. Weiner, I. Florin, D. Hellhammer and M. Murison (Eds.), *New Frontiers of Stress Research*, Hans Huber, 1988, pp. 1-11.
 - 240 Takahashi, L.K., Kalin, N.H., Vanden Burgt, J.A. and Sherman, J.E., Corticotropin-releasing factor modulates defensive-withdrawal and exploratory behavior in rats, *Behav. Neurosci.*, 103 (1989) 648-654.
 - 241 Taya, K. and Sasamoto, S., Inhibitory effects of corticotropin-releasing factor and β -endorphin on LH and FSH secretion in the lactating rat, *J. Endocrinol.*, 120 (1989) 509-515.
 - 242 Tazi, A., Dantzer, R., Le Moal, M., Rivier, J., Vale, W. and Koob, G.F., Corticotropin-releasing factor antagonist blocks stress-induced fighting in rats, *Regulat. Pept.*, 18 (1987) 37-42.
 - 243 Tazi, A., Swerdlow, N.R., Le Moal, M., Rivier, J., Vale, W. and Koob, G.F., Behavioral activation by CRF: evidence for the involvement of the ventral forebrain, *Life Sci.*, 41 (1987) 41-49.
 - 244 Udelsman, R., Harwood, J.P., Millan, M.A., Chrousos, G.P., Goldstein, D.S., Zimlichman, R., Catt, K.J. and Aguilera, G., Functional corticotropin releasing factor receptors in the primate peripheral sympathetic nervous system, *Nature*, 319 (1986) 147-150.
 - 245 Uehara, A., Sekiya, C., Takasugi, Y., Namiki, M. and Arimura, A., Anorexia induced by interleukin-1: involvement of corticotropin-releasing factor, *Am. J. Physiol.*, 257 (1989) R613-617.
 - 246 Vale, W., Spiess, J., Rivier, C. and Rivier, J., Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β -endorphin, *Science*, 213 (1981) 1394-1397.
 - 247 Valentino, R.J., Corticotropin-releasing factor: putative neurotransmitter in the noradrenergic locus coeruleus, *Psychopharmacol. Bull.*, 25 (1989) 306-311.
 - 248 Valentino, R.J. and Foote, S.L., Corticotropin-releasing factor disrupts sensory responses of brain noradrenergic neurons, *Neuroendocrinology*, 45 (1987) 28-36.
 - 249 Valentino, R.J. and Foote, S.L., Corticotropin-releasing hormone increases tonic but not sensory-evoked activity of noradrenergic locus coeruleus neurons in unanesthetized rats, *J. Neurosci.*, 8 (1988) 1016-1025.
 - 250 Valentino, R.J., Foote, S.L. and Aston-Jones, G., Corticotropin-releasing factor activates noradrenergic neurons of the locus coeruleus, *Brain Res.*, 270 (1983) 363-367.
 - 251 Valentino, R.J. and Wehby, R.G., Corticotropin-releasing factor: evidence for a neurotransmitter role in the locus coeruleus during hemodynamic stress, *Neuroendocrinol.*, 48 (1988) 674-677.
 - 252 Van Loon, G.R., Shum, A. and Ho, D., Lack of effect of corticotropin releasing factor on hypothalamic dopamine and serotonin synthesis turnover rates in rats, *Peptides*, 3 (1982) 799-803.
 - 253 Vanvugt, D.A., Webb, M.Y. and Reid, R.L., Naloxone antagonism of corticotropin-releasing hormone stimulation of prolactin secretion in rhesus monkeys, *J. Clin. Endocrinol. Metab.*, 68 (1989) 1060-1066.
 - 254 Veldhuis, H.D. and De Wied, D., Differential behavioral actions of corticotropin-releasing factor (CRF), *Pharmacol. Biochem. Behav.*, 21 (1984) 707-713.
 - 255 Webster, E.L. and De Souza, E.B., Corticotropin-releasing factor receptors in mouse spleen: identification, autoradiographic localization and regulation by divalent cations and guanine nucleotides, *Endocrinology*, 122 (1988) 609-617.
 - 256 Webster, E.L., Tracey, D.E., Jutila, M.A., Wolfe, S.A. and Desouza, E.B., Corticotropin-releasing factor receptors in mouse spleen - identification of receptor-bearing cells as resident macrophages, *Endocrinology*, 127 (1990) 440-452.
 - 257 Weiner, R.I. and Ganong, W.F., Role of brain monoamines and histamine in regulation of anterior pituitary secretion, *Physiol. Rev.*, 58 (1978) 905-976.
 - 258 Weiss, J.M. and Simson, P.E., Neurochemical and electrophysiological events underlying stress-induced depression in an animal model. In G.P. Chrousos, D.L. Loriaux and P.W. Gold (Eds.), *Mechanisms of Physical and Emotional Stress*, Plenum Press, New York, 1988, pp. 425-440.
 - 259 Weiss, S.R.B., Post, R.M., Gold, P.W., Chrousos, G., Sullivan, T.L., Walker, D. and Pert, A., CRF-induced seizures and behavior: interaction with amygdala kindling, *Brain Research*, 372 (1986) 345-351.
 - 260 Williams, C.L., Peterson, J.M., Villar, R.G. and Burks, T.F., Corticotropin-releasing factor directly mediates colonic responses to stress, *Am. J. Physiol.*, 253 (1987) G582-G586.
 - 261 Winslow, J.T., Newman, J.D. and Insel, T.R., CRH and α -helical-CRH modulate behavioral measures of arousal in monkeys, *Pharmacol. Biochem. Behav.*, 32 (1989) 919-926.
 - 262 Wynn, P.C., Hauger, R.L., Holmes, M.C., Millan, M.A., Catt, K.J. and Aguilera, G., Brain and pituitary receptors for corticotropin releasing factor: localization and differential regulation after adrenalectomy, *Peptides*, 5 (1984) 1077-1084.
 - 263 Wynn, P.C., Harwood, J.P., Catt, K.J. and Aguilera, G., Regulation of corticotropin-releasing factor (CRF) receptors in the rat pituitary gland: effects of adrenalectomy on CRF receptors and corticotroph responses, *Endocrinology*, 116 (1985) 1653-1659.
 - 264 Wynn, P.C., Harwood, J.P. and Catt, K.J., Corticotropin-releasing factor (CRF) induces desensitization of the rat pituitary CRF receptor-adenylate cyclase complex, *Endocrinology*, 122 (1988) 351-358.
 - 265 Yang, X.-M. and Dunn, A.J., Central β_1 -adrenergic receptors are involved in CRF-induced defensive withdrawal, *Pharmacol. Biochem. Behav.*, 36 (1990) 847-851.
 - 266 Yang, X.-M., Gorman, A.L. and Dunn, A.J., The involvement of central noradrenergic systems and corticotropin-releasing factor in defensive withdrawal in rats, *J. Pharmacol. Expt. Ther.*, in press.
 - 267 Yasuda, N., Greer, M.A. and Aizawa, T., Corticotropin-releasing factor, *Endocrine Rev.*, 3 (1982) 123-140.

ENP 00054

CSF corticotropin-releasing hormone and somatostatin in major depression: response to antidepressant treatment and relapse

Csaba M. Banki^a, Lajos Karmacs^a, Garth Bissette^b and Charles B. Nemeroff^c

^aRegional Neuropsychiatric Institute, Nagykallo, Hungary; ^bDuke University Medical Center, Department of Psychiatry, Durham, NC, USA; ^cEmory University School of Medicine, Department of Psychiatry, Atlanta, GA, USA

(Received 28 October, 1991)

(Accepted 22 January, 1992)

Key words: Corticotropin-releasing hormone; Somatostatin; Major depression; Cerebrospinal fluid; Relapse

Summary

Immunoreactive corticotropin-releasing hormone (CRH) and somatostatin (SRIF) were measured in the cerebrospinal fluid (CSF) of 24 female in-patients, suffering from DSM-III-R major depression, both before and after antidepressant treatment. In the total group there were no significant differences between pre- and post-treatment CSF-CRH and SRIF concentrations despite satisfactory clinical improvement in each patient. However, there was a significant post-treatment reduction of the CSF-CRH concentration in the 15 patients who remained depression-free for at least 6 months following treatment, in contrast to the tendency for elevation in those 9 subjects who relapsed within 6 months. CSF-SRIF showed no similar pattern. High, or even increasing, CSF-CRH concentration during antidepressant treatment may indicate lack of normalization of an underlying process in major depression despite symptomatic improvement and predicted early relapse.

Introduction

Neuropeptides have been thought to be involved in the biological pathogenesis of depressive disorders since their role as neurotransmitters, co-transmitters, or neuromodulators, has been recognized. Corticotropin-releasing hormone (CRH), a 41-amino acid peptide regulates corticotropin (ACTH) release from the pituitary and is also a putative neurotransmitter in various brain regions involved in the regulation of affective states and behavioral responses (Nemeroff, 1988; Koob and Bloom, 1985). Central CRH overproduction is one possible mechanism underlying the hypothalamic-pituitary-adrenal (HPA) hyperactivity seen in

many patients with major depression (Gold et al., 1984; Gold and Chrousos, 1985; Holsboer, 1988). In fact, elevated cerebrospinal fluid CRH concentrations were found in Swedish (Nemeroff et al., 1984), American (Bissette et al., 1985) and Hungarian (Banki et al., 1987) patients diagnosed as having major depression, although not all studies agreed (Roy et al., 1987). In one study, reduced CRH binding site number was also reported in the frontal cortex of 26 suicide victims as compared with 29 controls (Nemeroff et al., 1988). More recently a significant decrease of CSF-CRH concentration in nine depressed patients after receiving electroconvulsive treatment was reported (Nemeroff et al., 1991). Synthetic CRH elicits blunted ACTH response in many patients with major depression and the response usually normalizes with clinical recovery (Gold et al., 1984;

Correspondence to: Csaba M. Banki, MD, PhD, P.O. Box 37, H-4321 Nagykallo, Hungary. Tel.: (36-42) 63-133.

Holsboer, 1988). These data, concordant with earlier observations on other measures of HPA hyperactivity in depression, such as plasma or urinary cortisol and the dexamethasone suppression test (Halbreich et al., 1985; Carroll, 1985) suggest that central CRH overproduction may be a state-dependent phenomenon in at least a subgroup of patients with major depression. There are, however, very limited data on CRH changes during antidepressant drug treatment and on its possible predictive value for depressive relapse.

Somatostatin (somatotropin release-inhibiting factor = SRIF) is another neuropeptide widely distributed in the brain and probably acting as a neurotransmitter or a co-transmitter (Nemeroff et al., 1987). There is clinical evidence that SRIF concentrations in the CSF of depressed patients may be significantly reduced during the depressive phase and normalize after recovery (Rubinow et al., 1984; Ågren and Lundqvist, 1984; Post et al., 1988). However, SRIF reduction is not specific to depression but may occur in several neurological and psychiatric disorders (Loosen and Banki, 1988) where it may quite generally indicate the 'active' or 'acute' phases of the disease. HPA hyperactivity (dexamethasone nonsuppression) was found to be associated with low CSF-SRIF in both depressed and schizophrenic subjects (Doran et al., 1986), again suggesting that low CSF-SRIF was also a state-dependent neuropeptide marker. However, electroconvulsive treatment (ECT) caused only a weak, nonsignificant SRIF elevation in depressed patients (Nemeroff et al., 1991). The interrelationships between central SRIF-containing neurone systems and other neurotransmitters (catecholamines, GABA, HPA axis, etc.) and the observation that carbamazepine decreased CSF-SRIF concentrations (Post et al., 1988) make this neuropeptide an important putative mediator of at least some symptoms of depressive disorders. Data on the possible relationship of CSF-SRIF to clinical recovery and relapse have still remained scarce, however.

The present study was performed in severely ill, mostly psychotic depressed inpatients in order to demonstrate CSF neuropeptide changes after successful anti-depressant treatment. In addition, a 6-month follow-up was included to see if either baseline or treatment-induced neuropeptide changes can predict early relapse in major depression.

Patients and methods

Twenty-four female psychiatric in-patients, recently hospitalized in a regional Hungarian hospital specialized in psychiatry, were asked to participate in the study. Their physical and demographic characteristics are summarized in Table 1. All were diagnosed according to the DSM-III-R as having severe major depression; 15 had melancholic features, another 3 had psychotic features, and six had the global severity rating as 'severe' in the DSM-III-R. The large majority of the group had never had manic or hypomanic episodes in the past with the exception of 2 subjects (one bipolar-I and one bipolar-II, in RDC terms). They all gave written consent to participate, after a detailed explanation of the procedure, in accordance with the Helsinki declaration requirements. Pre-treatment examination included a detailed physical, neurological, and laboratory examination to rule out any significant medical illness, endocrine or neurological abnormality; in addition, we excluded individuals with alcohol or other psychoactive substance dependence, pregnancy or lactation, use of steroid drugs within 6 months, and other major psychiatric disorders such as dementia, mental retardation, or personality disorder. None of the patients reported use of antidepressants, neuroleptics, lithium, or anticonvulsant drugs within the last 2 weeks before admission.

After 2–5 days of initial evaluation and stabilization in the hospital environment, and following the obtaining of the written consent in the presence of two staff members, lumbar punctures were performed at 9:00–10:00 a.m. in a sitting position at the fourth intervertebral space. Ten ml CSF was obtained in a plastic tube and

TABLE 1
PHYSICAL AND BACKGROUND VARIABLES IN DEPRESSED PATIENTS

Values are expressed as mean \pm SD (range) where appropriate.

Age (years)	51.4 \pm 10.4 (23–70)
Weight (kg)	63.5 \pm 11.4 (39–80)
Height (cm)	154.9 \pm 6.6 (146–174)
No. episodes	2.5 \pm 1.4 (1–6) not including index episode
First onset (years)	6.2 \pm 7.5 (0.5–30) median 4 years
Episode duration (weeks)	5.3 \pm 3.7 (2–10) median 3 weeks
G.A.F. score (DSM-III-R)	33.0 \pm 10.0 (18–50)
HAMD score	37.1 \pm 5.3 (24–46)
Melancholic type	15/24 patients

immediately frozen at -70°C without any additional conservant. Before the LP patients received only 1–2 mg alprazolam or 300–600 mg chlormethiazole when necessary to control excessive anxiety or agitation or to promote sleep; LPs were performed after 12 h fasting and an overnight controlled bedrest. No major LP-related complaints were seen following the procedure apart from mild, transient headache in 5 subjects.

Antidepressant drug treatment was initiated after the LP according to routine hospital procedures. The principal drug was 125–225 mg maprotiline ($n = 15$), 480–720 mg dibenzepine ($n = 5$), 150–200 mg amitriptyline ($n = 4$); as adjuvants anxiolytics, thioxanthene neuroleptics were also used. The severity of depression was evaluated on the 24-point Hamilton Depression Scale (HAMD), and the Clinical Global Impression (CGI) scale was also used to measure severity and treatment change. In two patients no significant antidepressant response was achieved after 4 weeks and therefore they received a course of 4 and 6 ECT each (in methohexital anesthesia with succinylcholine relaxation, assisted respiration with oxygen, and cuff monitoring). All 24 patients improved significantly by the 5th to 8th week of treatment as indicated by at least 50% decrease of the HAMD score and at least 'much improved' on the CGI. Final HAMD scores were between 6 and 22, still indicating clinically significant depressive symptomatology in some patients ($n = 5$) while 19 others had no final HAMD rating above 14.

A second LP was performed in the 6th to 8th week of treatment, before discharge, when clinically significant improvement occurred; the procedure was the same as with the first LP but antidepressant drug treatment was not interrupted. All subjects remained under hospital supervision for at least 24 h following the LP.

All CSF samples were stored at -70°C in darkness for 1–3 months and then transported by air in a plastic container containing dry ice to the United States (Dr. Nemeroff, Psychoendocrine Laboratory, Duke University Medical Center) for peptide analysis. All samples were coded before transportation and the code was made available to the laboratory only after the last sample was analyzed. Both CSF-CRH and CSF-SRIF were analyzed using sensitive and specific immunoassay procedures described earlier (Nemeroff et al., 1984; Bissette et al., 1986).

All 24 patients were followed up regularly after discharge as outpatients, within the same psychia-

tric hospital. Despite continuing antidepressant medication and regular visits nine patients suffered a depressive relapse within the first six months, severe enough to require at least a few days' rehospitalization. The remaining 15 women remained depression-free during the 6-month follow-up period and they received a maintenance dose of their respective antidepressant medication continuously.

Pre- and post-treatment CSF neuropeptide data were compared with paired *t*-tests; the difference between relapsed and nonrelapsed groups was evaluated by analysis of covariance. Correlations with neuropeptide concentrations and background variables were computed by Pearson's coefficients, and their effect on the peptide concentration was calculated by multiple regression analysis. Baseline CRH and SRIF data were log-transformed for these calculations because they appeared to be log-normally distributed as observed earlier (Banki et al., 1987).

Results

Mean \pm SD of each variable, together with the range, is given in Table 2. where both ΔCRH and ΔSRIF are calculated as the difference between the post- and pre-treatment value (minus difference indicating decrease). Difference values appeared to be approximately normally distributed for both neuropeptides; the mean difference of CSF-CRH in the total group was -5.6 ± 29.0 ($t = 0.94$, d.f. = 23, N.S.), and the mean difference of CSF-SRIF was 3.3 ± 13.5 pg/ml ($t = 1.19$, d.f. = 23, N.S.).

Figures 1 and 2 present individual CSF-SRIF and CSF-CRH data pairs of patients, separately in the relapsed and nonrelapsed subgroups. While there was no significant difference in either subgroup and between the subgroups for the log-SRIF concentration before and after treatment

TABLE 2
MEANS AND STANDARD DEVIATIONS OF BASELINE NEUROPEPTIDES AND CHANGES DURING ANTIDEPRESSANT TREATMENT

	pg/ml
CRH	66.1 ± 38.8 (19–178)
ΔCRH	-5.6 ± 29.0 (–73–50) ^a
SRIF	27.8 ± 13.1 (8–51)
ΔSRIF	3.3 ± 13.5 (–22–37) ^a

^aCalculated as post-treatment minus pre-treatment difference.

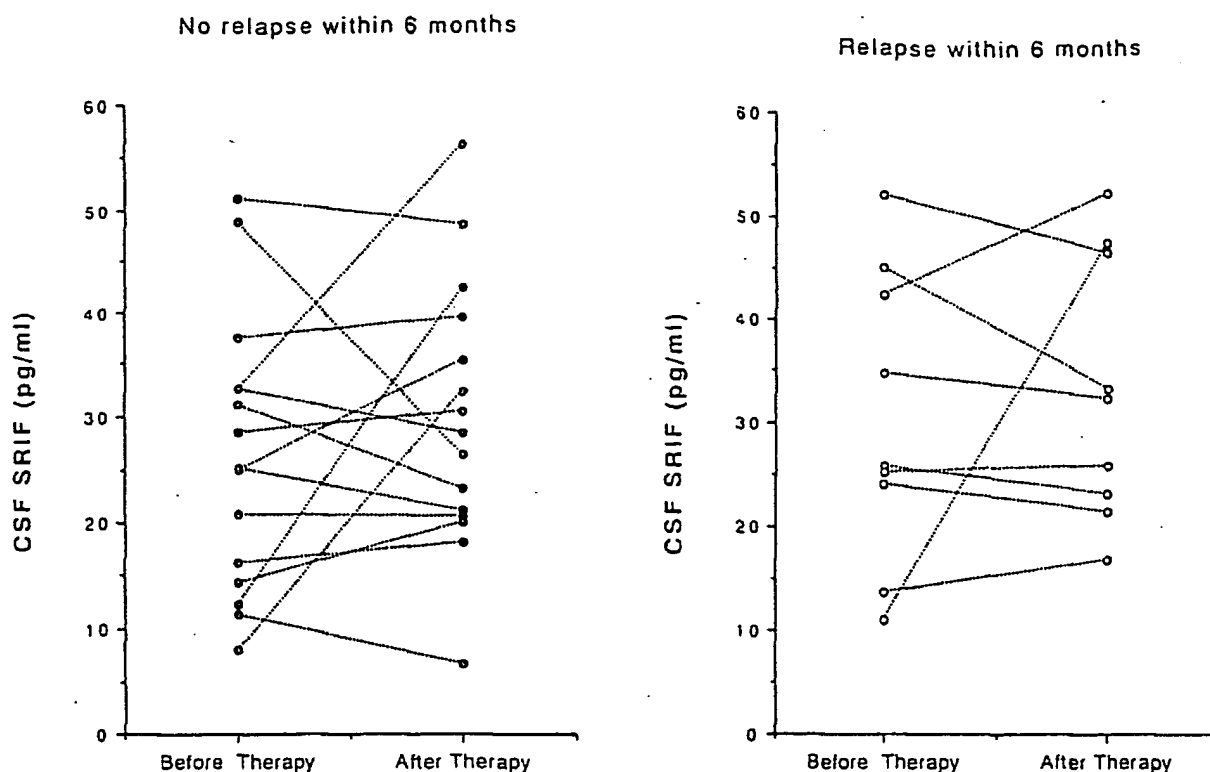


Fig. 1. CSF somatostatin changes during antidepressant treatment in patients with and without early relapse. The response was not different between the subgroups (ANCOVA $F(1,21) = 0.47$, $P > 0.50$, N.S.).

(ANCOVA, $F(1,22) = 0.47$, N.S.), there was a significant difference between the log-CRH responses of the subgroups (ANCOVA $F(1,21) = 8.83$, $P < 0.008$). As can be seen in Fig. 2, CSF-CRH values in the non-relapsed subgroup decreased significantly ($t = 2.58$, d.f. = 14, $P < 0.025$) while they tended to increase in the relapsed patients ($t = 1.48$, d.f. = 8, N.S.).

We registered several clinical variables in our patients and computed their correlations with both baseline and treatment-induced neuropeptide change values. The correlation coefficients, using log-transformation for CRH and SRIF, are presented in Table 3. It is noteworthy to emphasize the significant negative correlations of both baseline neuropeptide concentrations with body height, and the positive correlation of CSF-CRH with age. No other significant coefficient was found. Based on these variables we computed multiple regression analysis to see whether CSF neuropeptide concentrations were significantly dependent on these variables: none of these analyses resulted in significant R^2 -values (CRH: 0.601; Δ CRH: 0.512; SRIF: 0.465; Δ SRIF: 0.383).

The highest multiple determination coefficient was seen for baseline CSF-CRH and its only significant beta was for body height. However, the significance of these few findings among the large number of coefficients calculated for a small patient sample must remain uncertain.

There was no significant difference for any neuropeptide measurement between patient subgroups receiving different antidepressants; there

TABLE 3
CORRELATIONS BETWEEN NEUROPEPTIDE AND CLINICAL VARIABLES

	CRH	Δ CRH	SRIF	Δ SRIF
Age	0.41*	-0.24	0.31	0.05
Weight	-0.08	0.27	0.01	0.27
Height	-0.47*	0.10	-0.50*	0.21
No. episodes	-0.11	0.20	-0.02	-0.10
Time/onset	-0.06	0.37	0.05	-0.16
Epis. duration	-0.06	0.00	0.38	-0.36
G.A.F.	-0.23	0.11	0.08	-0.05
HAMD	0.23	-0.11	-0.06	0.10
Melancholia	0.23	0.09	-0.16	0.31

* $P < 0.05$.

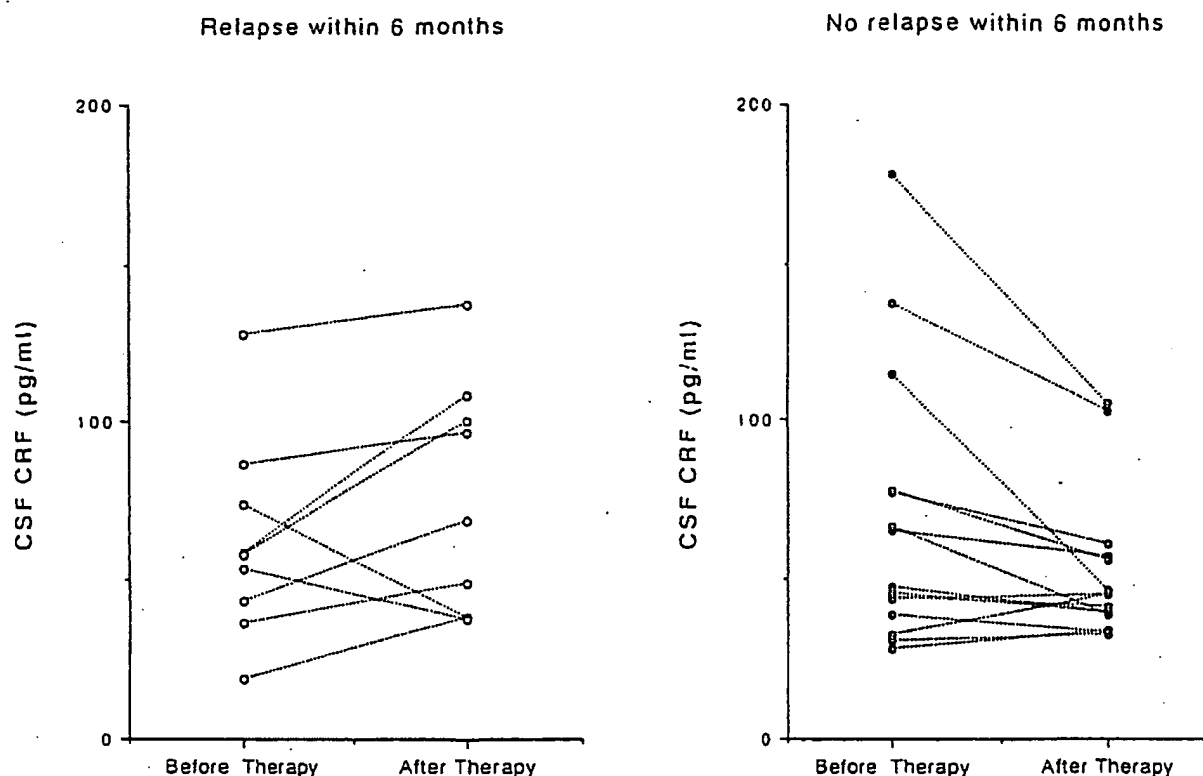


Fig. 2. CSF corticotropin-releasing hormone changes during antidepressant treatment in patients with and without early relapse. The response was significantly different between the subgroups (ANCOVA, $F(1,21) = 8.83$, $P < 0.008$).

was no difference for the 2 subjects who received ECT (neither of them relapsed within 6 months) as compared to those who received only drugs; and there was no difference between the melancholic and non-melancholic patients in their peptide responses. No correlation between the final HAMD score or the HAMD difference score and either CRH or SRIF responses were found. Finally, no correlation between duration of treatment (between 35 and 54 days) and CSF neuropeptide levels could be demonstrated.

Discussion

Mean baseline CSF-CRH concentration in this population of major, mostly melancholic and/or psychotic depressed women was above 66 pg/ml which is similar to our earlier observations (Banki et al., 1987). None of these patients has been included in any other previous reports, thus indicating reproducibility of the findings (and of the interassay characteristics of our CRH measurement procedure as well). In the discordant study by

Roy et al. (1987) the controls had remarkably similar mean (and also SD) values of CSF-CRH as our controls; the higher peptide concentrations in our depressed patients may be related to their more advanced age (which may be correlated to CSF-CRH), more severe depression, shorter hospitalization before LP, or less exposure to antidepressant drugs in the preceding weeks or months, but these explanations remain speculative in the absence of between-country comparative studies of depressed in-patients. Baseline SRIF concentrations were found to be somewhat lower than the mean values reported from other laboratories (Post et al., 1988) but still in the same range and with very similar standard deviation.

Both neuropeptide concentrations were found to be significantly correlated with body height. This phenomenon, not seen earlier (Banki et al., 1987) may be due to similar factors which underlie the same relationship seen with the monoamine metabolites in the CSF; in fact, there is a significant cranio-caudal gradient for CSF-CRH (Arato et al., 1989) and probably for SRIF as well (Rubinow et al., 1984). The positive correlation

between CSF-CRH and age may be limited to depressed women, or to an older age group, because it was not seen earlier in controls and it was not seen in other patient populations (Nemeroff et al., 1984; Roy et al., 1987). Our specific population excluded the investigation of sex differences.

The most important finding was the absence of significant reduction in CSF-CRH concentration in the total group despite the clinically significant, marked symptomatic improvement. This is different from the short-time effect of ECT on CSF-CRH (Nemeroff et al., 1991) and indicates a dissociation between the neuropeptide release into the CSF and the presence of psychological symptoms of depression. A possible confounding factor is the largely unknown direct effect of antidepressant drugs on central CRH release; but it is noteworthy that there was no difference between the effects of a selective noradrenaline reuptake blocker (maprotiline) and the nonselective drugs (dibenzepine and amitriptyline) on the CSF-CRH. This may indicate that CSF-CRH changes are more associated with the drug-induced longterm adaptive processes than with the immediate reuptake blocking. The other major finding in this study, namely the lack of reduction of CSF-CRH levels being predictive on an early depressive relapse, corroborate this assumption. It supports earlier findings with the dexamethasone suppression test (Greden et al., 1980; Carroll, 1982) where likewise normalization occurred only in a subgroup of patients but this was found to be a good prognostic sign. With the CRH/ACTH test, normalization with recovery was the rule (Gold et al., 1984) but it did not occur in each case; whether the non-normalization predicts relapse needs confirmation in larger patient samples. There is some recent evidence from preclinical work that chronic glucocorticoid treatment may induce catecholaminergic and serotonergic changes both pre- and post-synaptically that resemble changes assumed to develop in human depression (Szemerédi et al., 1988; Bagdy et al., 1989; Calogero et al., 1990). Along this line it can be hypothesized that an underlying HPA overactivity (of which CSF-CRH concentration may be one measure) represents a primary abnormality while monoaminergic changes that are directly influenced by antidepressant drugs are 'secondary' phenomena more closely associated with the psychological symptoms. In this respect the more uniform response of CRF-CRH to ECT corresponds to its different mechan-

ism of action (Fink and Ottoson, 1980) in particular on the peptidergic processes.

We did not observe a significant elevation, ie. normalization, of CSF-SRIF during antidepressant drug treatment. This was absent both in the relapsing and the nonrelapsing patients. Remarkably, no significant SRIF elevation was found after ECT either (Nemeroff et al., 1991). Earlier studies found 'normal' mean SRIF levels in affective patients in the euthymic state but also described no effect of common antidepressants on the CSF-SRIF concentration (Rubinow et al., 1984). Selective serotonergic antidepressants may elevate SRIF while carbamazepine may decrease it, but we used neither drugs in this study. Whether normalization occurs after a longer euthymic period remains to be investigated.

In summary, we found no consistent changes of either CSF-CRH or CSF-SRIF in response to a clinically successful antidepressant drug treatment during 5–8 weeks. However, patients who relapsed within 6 months could be separated by their non-decreasing CSF-CRH concentrations from those who remained symptom-free and showed significant reduction of their initially elevated CRH values during treatment. Although CSF neuropeptide measurements are unlikely to become common tools in psychiatric practice they represent an important research tool to understand the underlying pathophysiology of major depression.

References

- Ågren, H. and Lundqvist, G. (1984) Low levels of somatostatin in human CSF mark depressive episodes. *Psychoneuroendocrin.* 9, 233–248.
- Arato, M., Banki, C.M., Bissette, G. and Nemeroff, C.B. (1989) Elevated CSF CRF in suicide victims. *Biol. Psychiat.* 25, 355–359.
- Bagdy, G., Calogero, A.E., Chrousos, G.P. and Szemeredy, K. (1989) Delayed effects of chronic cortisol treatment on brain and plasma concentrations of corticotropin (ACTH) and beta-endorphin. *Brain Res.* 489, 216–222.
- Banki, C.M., Bissette, G., Arato, M., O'Connor, L. and Nemeroff, C.B. (1987) CSF corticotropin-releasing factor-like immunoreactivity in depression and schizophrenia. *Am. J. Psychiat.* 144, 873–877.
- Bissette, G., Spielman, F., Stanley, M., Banki, C.M., Fink, M., Stanley, B., Golden, R.I. and Nemeroff, C.B. (1985) Further studies of corticotropin-releasing factor-like immunoreactivity in patients with affective disorders. *Soc. Neurosci. Abstr.* 11, 133.
- Bissette, B., Widerlöv, E., Walleus, H. and Nemeroff, C.B. (1986) Alterations in CSF concentrations of somatostatin-like immunoreactivity in neuropsychiatric disorders. *Arch. Gen. Psychiat.* 43, 1148–1154.

- Calogero, A.E., Bagdy, G., Szemeredy, K., Tartaglia, M.E., Gold, P.W. and Chrousos, G.P. (1990) Mechanism of serotonin receptor agonist-induced activation of the hypothalamic-pituitary-adrenal axis in the rat. *Endocrinology* 126, 1888-1894.
- Carroll, B.J. (1982) The dexamethasone suppression test for melancholia. *Br. J. Psychiat.* 140, 292-304.
- Carroll, B.J. (1985) Dexamethasone suppression test: a review of contemporary confusion. *J. Clin. Psychiat.* 46, 13-24.
- Doran, A.R., Rubinow, D.R., Roy, A. and Pickar, D. (1986) CSF somatostatin and abnormal response to dexamethasone administration in schizophrenia and depressed patients. *Arch. Gen. Psychiat.* 43, 365-369.
- Fink, M. and Ottoson, J.O. (1980) A theory of convulsive therapy in endogenous depression: significance of hypothalamic functions. *Psychiat. Res.* 2, 49-61.
- Gold, P.W., Chrousos, G., Kellner, C., Post, R., Roy, A., Augerinos, P., Oldfield, E. and Loriaux, D.L. (1984) Psychiatric implications of basic and clinical studies with corticotropin-releasing factor. *Am. J. Psychiat.* 141, 619-627.
- Gold, P.W. and Chrousos, G.P. (1985) Clinical studies with corticotropin-releasing factor: implications for the diagnosis and pathophysiology of depression, Cushing's disease, and adrenal insufficiency. *Psychoneuroendocrinology* 10, 401-419.
- Greden, J.P., Albala, A., Haskett, R.F. and Carroll, B.J. (1980) Normalization of dexamethasone suppression test: a probable index of recovery among endogenous depressives. *Biol. Psychiat.* 15, 449-458.
- Halbreich, U., Asnis, G.M., Schindlacker, R., Zumoff, B. and Nathan, R.S. (1985) Cortisol secretion in endogenous depression. *Arch. Gen. Psychiat.* 42, 904-908.
- Holsboer, F. (1988) Implications of altered limbic-hypothalamic-pituitary-adreno-cortical (LHPA) function for neurobiology of depression. *Acta Psychiat. Scand.* 77 (Suppl. 341), 72-111.
- Koob, G.F. and Bloom, F.E. (1985) Corticotropin-releasing factor and behavior. *Fed. Proc.* 44, 259-263.
- Loosen, P.T. and Banki, C.M. (1988) The use of nonopioid neuropeptides as diagnostic tools in psychiatric and neurological disorders. In: Nemeroff, C.B. (Ed.), *Neuropeptides in Psychiatric and Neurological Disorders*, Johns Hopkins Univ. Press, Baltimore, MD, pp. 18-48.
- Nemeroff, C.B., Widerlöv, E., Bissette, G., Walleus, H., Carlsson, I., Eklund, K., Kilts, C.D. Loosen, P.T. and Vale, W. (1984) Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* 226, 1342-1344.
- Nemeroff, C.B., Walsh, T.J. and Bissette, G. (1987) Somatostatin and behavior: preclinical and clinical studies. In: Reichlin, S. (Ed.), *Somatostatin*, Plenum, New York, pp. 157-167.
- Nemeroff, C.B., Owens, M.J., Bissette, G., Andorn, A.C. and Stanley, M. (1988) Reduced corticotropin-releasing factor binding sites in the frontal cortex of suicide victims. *Arch. Gen. Psychiat.* 45, 577-579.
- Nemeroff, C.B. (1988) The role of corticotropin-releasing factor in the pathogenesis of major depression. *Pharmacopsychiatry* 21, 76-82.
- Nemeroff, C.B., Bissette, G., Akil, H. and Fink, M. (1991) Neuropeptide concentrations in the CSF of depressed patients treated with electroconvulsive therapy. *Br. J. Psychiat.* 158, 59-63.
- Post, R.M., Rubinow, D.R. and Gold, P.W. (1988) Neuropeptides in manic-depressive illness. In: Nemeroff, C.B. (Ed.), *Neuropeptides in Psychiatric and Neurological Disorders*, Johns Hopkins Univ. Press, Baltimore, MD, pp. 76-115.
- Roy, A., Pickar, D., Paul, S., Doran, A., Chrousos, G.P. and Gold, P.W. (1987) CSF corticotropin-releasing hormone in depressed patients and normal control subjects. *Am. J. Psychiat.* 144, 641-645.
- Rubinow, D.R., Gold, P.W., Post, R.M., Ballenger, J.C. and Cowdry, R.W. (1984) Somatostatin in patients with affective illness and in normal volunteers. In: Post, R.M. and Ballenger, J.C. (Eds.), *Neurobiology of Mood Disorders*, Williams and Wilkins, Baltimore, MD, pp. 369-387.
- Szemeredy, K., Bagdy, G., Stull, R., Calogero, A.E., Kopin, I.J. and Goldstein, D.S. (1988) Sympathoadrenomedullary inhibition in chronic glucocorticoid treatment in conscious rats. *Endocrinology* 123, 2585-2590.



Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated

Astrid W. Zobel, Thomas Nickel, Heike E. Künzel, Nibal Ackl, Annette Sonntag, Marcus Ising, Florian Holsboer*

Max Planck Institute of Psychiatry, Kraepelinstr. 2-10, D-80804 Munich, Germany

Received 1 March 2000; received in revised form 20 April 2000; accepted 25 April 2000

Abstract

Clinical and preclinical data suggest that unrestrained secretion of corticotropin-releasing hormone (CRH) in the CNS produces several signs and symptoms of depression and anxiety disorders through continuous activation of CRH₁ receptors. This led to the development of drugs that selectively antagonize CRH₁ receptors suppressing anxiety-like behavior in rats and also in monkey models of anxiety. These findings led to a clinical development program exploring the antidepressive potential of R121919, a water-soluble pyrrolopyrimidine that binds with high affinity to human CRH₁ receptors and is well absorbed in humans. This compound was administered to 24 patients with a major depressive episode primarily in order to investigate whether its endocrine mode of action compromises the stress-hormone system or whether other safety and tolerability issues exist. The patients were enrolled in two dose-escalation panels: one group ($n = 10$) where the dose range increased from 5–40 mg and another group ($n = 10$) where the dose escalated from 40 to 80 mg within 30 days each. Four patients dropped out because of withdrawal of consent to participate (three cases) or worsening of depressive symptomatology in one case. We found that R121919 was safe and well tolerated by the patients during the observation period. Moreover, the data suggested that CRH₁-receptor blockade does not impair the corticotropin and cortisol secretory activity either at baseline or following an exogenous CRH challenge. We also observed significant reductions in depression and anxiety scores using both, patient and clinician ratings. These findings, along with the observed worsening of affective symptomatology after drug discontinuation, suggests that the pharmacological principle of CRH₁-receptor antagonism has considerable therapeutic potential in the treatment and the prevention of diseases where exaggerated central CRH activity is present at baseline or following stress exposure. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Corticotropin-releasing hormone (CRH) has been identified as a neuropeptide that plays a central role in the coordination of neuroendocrine, autonomic and behavioral responses to stress (Vale et al., 1981). Once released from the hypothalamic paraventricular nucleus it enters the portal vessels via the median eminence to stimulate synthesis of proopiomelanocortin,

the precursor of corticotropin (ACTH). In response to stress exposure this neuropeptide hormone is secreted into the circulation and stimulates the synthesis and release of adrenal corticosteroids which in turn suppress the synthesis of both hypothalamic CRH and corticotrophic ACTH in order to reinstate homeostasis of the hypothalamic–pituitary adrenocortical (HPA) system (Plotsky, 1991). The setpoint which defines an individual's HPA homeostasis is determined by genetic as well as environmental factors, particularly traumatic events in early life (Coplan et al., 1996). If the setpoint is at a high level, negative feedback control upon hypothalamic CRH synthesis and release is decreased.

* Corresponding author. Tel.: 089-30622-220; fax: 089-30622-483.
E-mail address: Holsboer@mpipsykl.mpg.de (F. Holsboer).

This results in a continuous hyperactivity of CRH neural circuits directly or indirectly interconnecting the hypothalamic paraventricular nuclei (PVN) with extra-hypothalamic sites supposed to play an important role in the mediation of behavioral response to stress (for a review see Keck and Holsboer, 2000). For example, the locus coeruleus, a brainstem nucleus from which noradrenergic neurons project to the forebrain, contains CRH immunoreactive fibers and is activated by CRH (Valentino et al., 1993). Also, the central amygdala thought to mediate fear and anxiety (Davis, 1992), is innervated by CRH nerve terminals; and in this brain region in rodents CRH gene expression is thought to be activated by corticosteroids (Schulkin et al., 1998). Thus, impaired negative feedback by corticosteroids enhances release of CRH from the PVN which further increases corticosteroids via elevated ACTH. At the level of the amygdala this elevation of corticosteroids may even enhance CRH gene expression, possibly producing anxiety-like behavior. Other behavioral phenomena associated with unrestrained CRH include disturbed sleep, loss of sexual drive, psychomotor and autonomic changes and decreased appetite. These behavioral changes can also be induced by central administration of CRH in rats and monkeys (Kalin, 1985) or by inserting a CRH gene in the mouse genome resulting in CRH overproducing transgenic mice (Stenzel-Poore et al., 1994). All of the CRH-elicited effects resemble the clinical signs and symptoms characteristic for patients with severe depression (Owens and Nemeroff, 1991; Holsboer et al., 1992). These patients also show hyperactivity of the HPA system and experimental evidence has shown that a hyperactive CRH system is a major cause for this neuroendocrine disturbance. Clinical studies have also demonstrated elevated baseline ACTH and cortisol secretion, and their inadequate suppression by dexamethasone, a synthetic glucocorticoid (for a review see Holsboer, 1995). Further, CRH concentrations are elevated in the cerebrospinal fluid of depressed patients (Nemeroff et al., 1984), which, if extrapolated to the situation in the brain, is consistent with reduced CRH binding in forebrains of depressed suicide victims (Nemeroff et al., 1988) and elevated numbers of CRH-producing neurons in the PVN of patients with depression (Raadsheer et al., 1994). Finally, the ACTH response to human and ovine CRH was found to be blunted among depressed patients indicating desensitized CRH receptors secondary to central hypersecretion (Gold et al., 1984; Holsboer et al., 1984, 1986). These clinical data, and the behavioral data derived from manipulations of the CRH system in animals, are consistent with exaggerated CRH secretion as a causal mechanism accounting not only for the neuroendocrine but also for psychopathological symptoms of depression and anxiety dis-

orders (Holsboer, 1999). Since the CRH signal is mediated through different CRH receptors localized in different regions in the rat, mouse and human brain (Chalmers et al., 1996), it was important to know which of these two identified CRH receptors would be an appropriate target for a drug reducing the potentially depressogenic and anxiogenic effects of CRH. Studies using antisense oligodeoxynucleotides directed against the mRNA of CRH₁ and CRH₂ receptors, as well as mouse mutants where CRH₁ receptors were lacking, supported the hypothesis that stress-induced anxiety-like behavior is mediated through the CRH₁ receptors (Liebsch et al., 1995, 1999; Heinrichs et al., 1997; Skutella et al., 1994, 1998; Smith et al., 1998; Timpl et al., 1998; Steckler and Holsboer, 1999). This concept led pharmaceutical companies to screen compound libraries for molecules that might act as CRH₁ receptor antagonists and have properties suitable for clinical drug use (Chen et al., 1993; Schulz et al., 1996; Grigoriadis and de Souza, 1998; Shaham et al., 1998). One of these candidates is R121919 (formerly NBI 30775), a pyrrolopyrimidine, which is well absorbed when given orally, penetrates the blood-brain barrier and binds to cloned human CRH₁ receptors with high affinity ($K_i < 3$ nM) — binding to other neurotransmitter and neuropeptide receptors or transporters was absent or greater than 1000-fold different. Given subcutaneously to rats this compound antagonizes several behavioral effects induced by CRH pretreatment or by CRH overexpression in transgenic mice (Steckler et al., unpublished observation). In rats selectively bred for high anxiety-like behavior (Liebsch et al., 1998) the CRH₁-receptor antagonist R121919 blocked CRH binding to CRH₁ receptors and exerted anxiolytic effects in a dose-dependent manner in these rats (Keck et al., unpublished observation). Comparable anxiolytic effects of R121919 were absent in rats that were selectively bred for low anxiety. These preclinical observations prompted us to conduct an open-label trial in patients with major depression to get some initial information as to how this class of compounds might affect neuroendocrine and safety parameters and whether it can be well tolerated in this clinical condition. Although designed as a safety and tolerability study, not allowing definitive conclusions about efficacy to be made, we were also interested to observe whether specific changes in psychopathology might emerge during treatment with R121919.

2. Methods

Over the course of 13 months (December 1998 to December 1999) 24 patients were subsequently selected from referrals to the Clinical Department of the Max Planck Institute of Psychiatry for treatment of a major

depressive episode and enrolled into the study, provided they fulfilled the inclusion criteria. The study was conducted according to the regulations of the state of Bavaria (Federal Republic of Germany) and the Declaration of Helsinki which includes approval of the local ethical committee. Written informed consent was collected from all patients after the purpose and the experimental details and risks were explained and before protocol-specified procedures were initiated (see Fig. 1). Patients were informed that they could withdraw their consent at any time during the study without any justification and that the privacy of their medical records not related to the study protocol would be protected. Patients were required to have scores equal to or above 18 on the 21-item Hamilton Depression Rating Scale (HAMD) at screening and at day 0. Other exclusion criteria were a history or suspicion of substance abuse, specifically of alcohol, benzodiazepines, barbiturate, amphetamine and narcotics.

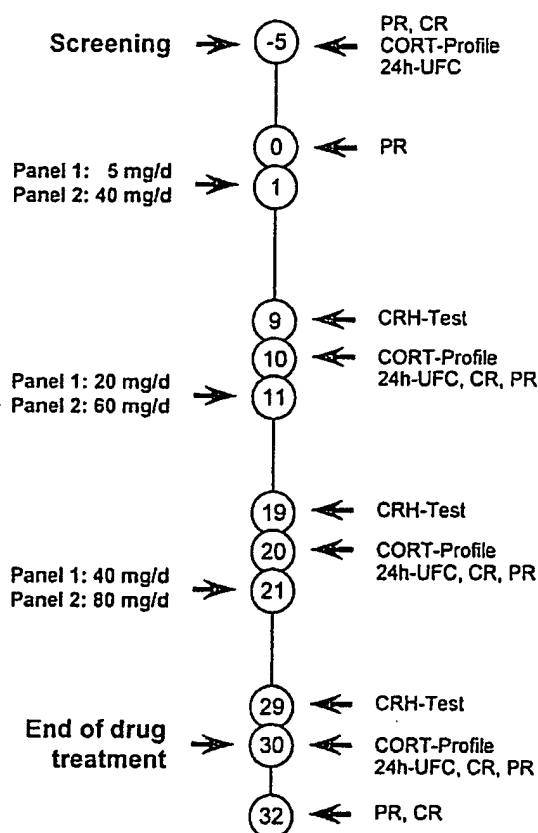


Fig. 1. Study design of both panels which only differ in the dose escalations. Abbreviations: PR=psychopathology rating (HAMD, HAMA, CGI, BDI, STAI); CR=clinical routine; CORT profile=venous blood samples taken at 0, 3, 8, 12 and 24 h for plasma ACTH and cortisol analysis; 24 h UFC=urinary free cortisol analysis in a 24-h urine collection.

Patients were not admitted if they had a serious or unstable medical illness including cardiovascular, rheumatoid, hepatic, renal, respiratory, metabolic, neurological or hematological disease. Specifically excluded were patients with endocrine disorders such as clinical or laboratory evidence for thyroid disease, Cushing's syndrome or Addison's disease. Women of child-bearing age were excluded even if contraceptive means were applied. Patients who posed a current suicidal risk were excluded, as were patients who were judged as treatment resistant, arbitrarily defined as non-responding to standard antidepressants at therapeutic dosages for at least 6 months. During the screening period all psychoactive medication was stopped for a minimum of 5 days and only chloralhydrate up to 2×500 mg/day was allowed as a sleeping aid. Patients having taken drugs known to be hepatic enzyme inducers, i.e. carbamazepine, barbiturates, phenytoin, cimetidine etc., were not enrolled. Pretreatment with monoamine oxidase inhibitors, fluoxetine or slow-release neuroleptics had to be discontinued for at least 1 month and treatment with corticosteroids or electroconvulsion within 3 months prevented study inclusion. Following screening, when all inclusion and exclusion criteria were defined, active treatment was initiated applying the two different dose-escalation regimens. For safety reasons the first 10 patients were recruited into panel 1 administering the lower dosages before panel 2 was started. Details of the dose escalation and the time points when psychopathometric assessments, clinical examinations and neuroendocrine tests were applied are illustrated in Fig. 1.

The laboratory analysis of clinical chemistry, hematology and hormone data was performed according to standard procedures. The circadian hormone secretion was estimated by ACTH and cortisol analysis in blood specimens at the time points given in Fig. 1. Total 24-h cortisol secretion was assessed by measuring free cortisol in a 24-h urine sample (UFC). Dose regimens for both panels are depicted in Fig. 1. The CRH test employed a protocol that was similar to that previously described (Holsboer et al. 1986): at 1400 h an indwelling catheter was inserted into a forearm vein and kept patent by a slow drip (50 ml/h) of physiological saline solution. At 14.00 h, 14.30 h, 14.45 h and 15.00 h a 3-ml venous blood sample was collected to assess baseline HPA activity. At 15.00 h a bolus of 100 µg of human CRH (Ferring, Germany) was injected through the catheter and samples were collected at the time points given in Fig. 2.

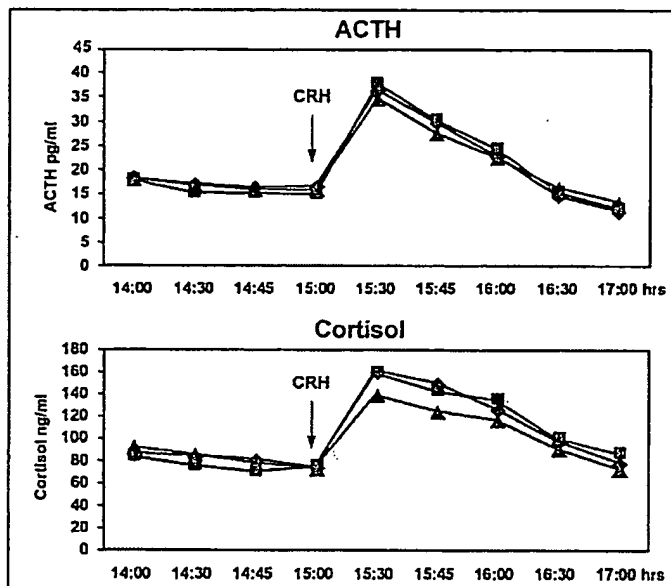
2.1. Data analysis

Differences within and between both dosing panels were assessed by methods based on analysis of variance. Within each panel the degree of

changes in symptom severity between screening and the consecutive days when tested under treatment [i.e. HAMD, Beck Depression Inventory (BDI), Hamilton Scale for Anxiety (HAMA), State Trait Anxiety Inventory (STAI), and Clinical Global Impression (CGI)] was estimated by deviation con-

trasts. These measures correspond to two-tailed paired *t*-test analyses. Because of the small sample size non-parametric Kendall's Tau coefficients were applied for correlational analyses. Construct-homogeneous correlations (i.e. depression related: HAMD, BDI; anxiety related: HAMA, STAI) were compared

Panel 1



Panel 2

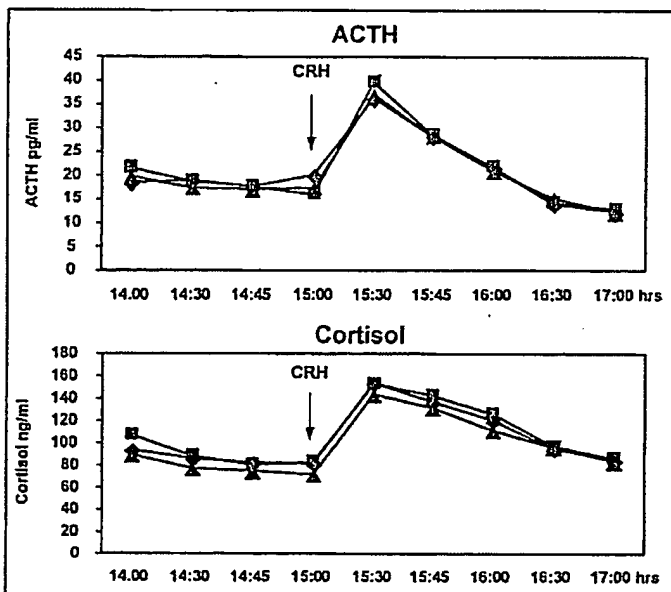


Fig. 2. Time-course curves of plasma ACTH and cortisol concentrations prior to and following an intravenous injection of 100 µg human CRH at 15:00 h at day 9 (diamond), day 19 (square) and day 29 (triangle) during panel 1 and panel 2. If CRH-stimulated plasma ACTH and cortisol output is expressed as the area under the time-course curve (AUC), no suppressive effect of R121919 on AUC values is noted at any time point at any dose given.

with construct-divergent correlations (HAMD/STAI and HAMA/BDI).

Effects of drug treatment upon neuroendocrine parameters employed deviation contrasts to assess the degree of change in hormonal secretions among patients treated in both panels. Analysis of effects of the drug upon circadian rhythmicity used a quadratic trend coefficient, reflecting the U-shaped circadian rhythm. The area under the time-course curve (AUC) for ACTH and cortisol, following CRH injections, was calculated according to a trapezoidal rule, and from this value the mean of cortisol levels calculated from the plasma ACTH and cortisol concentrations prior to CRH injection was subtracted. Again for correlational analyses, non-parametric Kendall's Tau coefficients were applied. But, under the perspective of outcome prediction, non-parametric Spearman's Rho coefficients were used because they are likely to be more appropriate in the case of a dichotomous outcome, which is treatment response vs treatment nonresponse. As neuroendocrine predictor variables urinary free cortisol (UFC), mean plasma cortisol, and ACTH concentration at screening day, and AUCs of ACTH and

cortisol following CRH infusion at day 9 were employed (see Fig. 1).

The data analysis presented here was based upon 20 patients that had completed the study. We also inspected what effect the inclusion of drop-out patients would have had if they were not prematurely discharged from the study and if their clinical condition at the time of drop-out would not have changed until termination of the study. This data analysis used the intent-to-treat approach and is presented in order to rule out the possibility that the differences between both dose-escalation panels were merely due to the drop-out cases in panel 2.

3. Results

3.1. Patients description

As documented in Table 1, there were no major differences between the patients in panel 1 and panel 2 with regard to gender, age, diagnostic attributions, length of index episode, family history and pretreatment. This allows comparison of drug effects regarding

Table 1

Demographic and clinical data of 20 completers (10 in panel 1 and 10 in panel 2) and of four patients who dropped out from panel 2 before the end of the trial. Patient (BDI and STAI) and clinician (HAMD, HAMA and CGI) rating scores before and after treatment with R121919 are also given (mean \pm SD). Symptoms are listed which worsened among six patients in both panels after discontinuation of the drug (see also Table 4)

	Panel 1 (10 patients)	Panel 2 (10 patients)	Drop out (4 patients)
Sex	6 males 4 females	5 males 5 females	2 males 2 females
Age	43.8 \pm 11.4	50.7 \pm 13.0	36.8 \pm 10.3
DSM IV 296.22	–	1 patient	–
DSM IV 296.23	3 patients	2 patients	1 patient
DSM IV 296.32	4 patients	3 patients	3 patients
DSM IV 296.33	3 patients	4 patients	–
Index episode (weeks)	18.2 \pm 13.7	18.0 \pm 6.6	23.5 \pm 19.6
Positive family history	8 patients	8 patients	2 patients
Pretreated with antidepressants	7 patients	7 patients	2 patients
HAMD screening	26.2 \pm 3.6	27.6 \pm 7.4	23.3 \pm 6.0
HAMD last day of medication	15.4 \pm 9.0	11.0 \pm 8.0	21.0 \pm 8.1 ^a
BDI screening	27.1 \pm 8.8	27.2 \pm 11.2	21.0 \pm 10.0
BDI last day of medication	21.1 \pm 10.1	12.9 \pm 6.2	22.8 \pm 6.7 ^a
HAMA screening	23.0 \pm 4.1	26.6 \pm 9.8	21.0 \pm 5.7
HAMA last day of medication	13.9 \pm 10.5	10.1 \pm 9.5	18.8 \pm 5.6 ^a
STAI screening	61.6 \pm 10.2	65.1 \pm 8.9	55.5 \pm 12.0
STAI last day of medication	57.8 \pm 14.8	51.8 \pm 8.6	63.0 \pm 5.9 ^a
CGI screening	3.7 \pm 0.5	4.1 \pm 1.0	3.75 \pm 0.5
CGI last day of medication	2.8 \pm 0.9	2.2 \pm 1.0	3.25 \pm 1.0 ^a
Remitter HAMD \leq 8	3 patients	6 patients	–
Responder)HAMD \geq 50%	5 patients	8 patients	–
Nonresponder	5 patients	2 patients	–
After cessation of medication worsening of symptoms	6 patients (agitation, mood, sleep, somatic symptoms)	6 patients (agitation, mood, sleep, somatic symptoms)	–

^a Psychopathology ratings of drop outs were derived from the last visit carry over approach.

safety and tolerability measures as well as psychopathometric scores.

3.2. Laboratory tests

Clinical chemistry, hematology ECG and EEG recordings yielded no adverse effects that could be specifically attributed to R121919. None of the 10 patients enrolled in panel 1 had elevated liver enzyme values during treatment with R121919. However, when shifted to mirtazapine, in five of these 10 patients liver enzyme values increased. In panel 2 we observed slightly increased liver transaminases during treatment with R121919 in three cases. The maximum values

reached in panel 2 were: AST: 45 U/l (normal range: 0–18 U/l); ALT: 80 U/l (normal range: 0–22 U/l); and GGT: 47 U/l (normal range: 6–28 U/l). After discontinuation of R121919 and the subsequent treatment of the patients with mirtazapine the liver enzyme values normalized in one patient and further increased in two other cases. In none of the cases did R121919 induce elevations of bilirubine. Heart rate tended to decrease during treatment in both panels and a small rebound was noted after drug cessation, however, no significant differences in heart rate between both panels emerged.

3.3. Endocrine effects

Comparison of UFC values yielded no significant difference between both panels at any time point. When UFC values during treatment were compared with UFC values at screening, a trend towards a steeper decline of UFC values was found in panel 2, where significant differences emerged at day 20, while significant differences in panel 1 were not observed until day 30.

In both dose-escalation panels no significant effects of the drug upon plasma cortisol, ACTH and renin concentrations were observed at any time. Moreover, no effects of the drug upon circadian rhythms of ACTH and cortisol emerged. When the plasma cortisol concentrations of all five sampling times were averaged, a trend for reduced mean cortisol values was observed across both panels. However, this cortisol lowering effect was not different between the two panels. Such trends were also not observed on plasma ACTH measurements. In addition, the CRH-elicited plasma ACTH and cortisol secretions were not different between both panels at any time point (see Fig. 2).

3.4. Psychopathometric findings

As illustrated in Fig. 3 HAMD rating scores dropped significantly in both panels. When these were analysed separately the differences between the mean HAMD values at each time point compared with the values at screening (baseline) were more marked in panel 2 (see also Tables 1 and 2). When a reduction of the HAMD score of $\leq 50\%$ of the score at screening was defined as criterion for response, five patients in panel 1 were identified as responders, three of whom were also remitters (i.e. they had a HAMD score of ≤ 8 points at day 30). In panel 2, eight out of 10 patients met the criterion of response, and six of these were also remitters (see Table 1). Notably, four patients of the total group of 14 enrolled in panel 2 dropped out before completing the 30-day study because of withdrawal of consent to participate in three cases at study days 13, 19 and 20, and a worsening of depressive symptomatology, including current

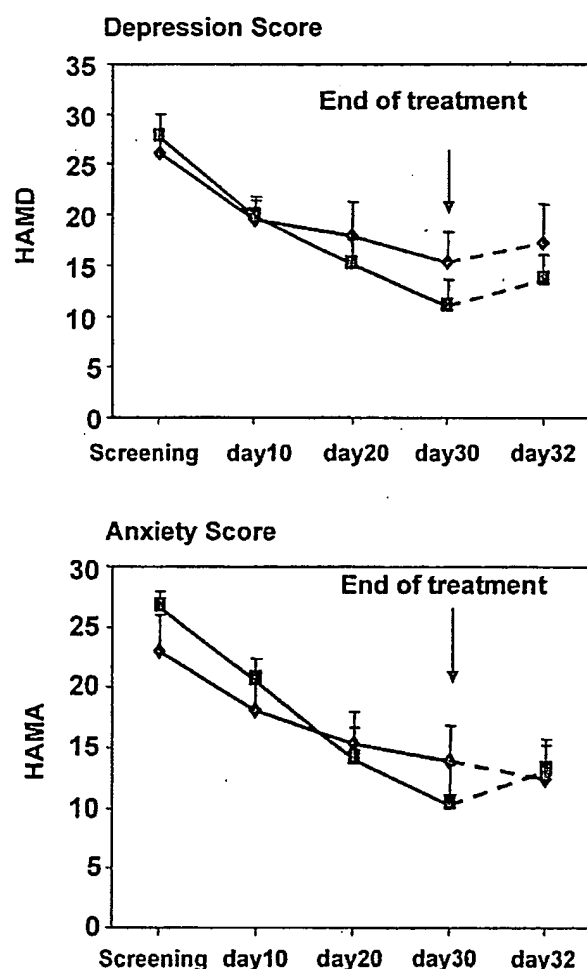


Fig. 3. Changes of HAMD and HAMA rating-scale scores in panel 1 (diamond) and panel 2 (square) show significant reductions in both panels and markedly better improvements in panel 2. In both panels HAMD rating-scale scores worsened after drug discontinuation. The HAMA rating-scale scores show increases after drug discontinuation in panel 2, but not in panel 1.

Table 2

Changes of rating-scales scores relative to screening during both panels. Note that absolute score differences (i.e. value at screening minus value at day of treatment) decreased more markedly in panel 2 than in panel 1. If the drop-out cases would have been included in panel 2 according to an intent-to-treat procedure, the HAMD mean \pm SEM (p) changes would have been: day 10: 6.8 ± 1.37 ($p < 0.001$); day 20: 9.5 ± 2.19 ($p = 0.001$); and day 30: 12.5 ± 2.36 ($p < 0.001$), suggesting that inclusion of the four drop-out cases would not have invalidated the overall conclusions

Panel 1 (<i>n</i> = 10)					Panel 2 (<i>n</i> = 10)			
Depression								
Time (days)	HAMD		BDI		HAMD		BDI	
	M ± SEM	<i>p</i>	M ± SEM	<i>p</i>	M ± SEM	<i>p</i>	M ± SEM	<i>p</i>
Screening								
–day 10	6.7 ± 2.07	0.010	1.5 ± 2.34	0.537	7.7 ± 1.52	0.001	6.6 ± 3.16	0.066
–day 20	8.3 ± 3.07	0.024	3.9 ± 2.86	0.206	12.4 ± 2.43	0.001	9.2 ± 4.23	0.058
–day 30	10.8 ± 2.72	0.003	6.0 ± 2.76	0.058	16.6 ± 2.05	0.001	14.3 ± 3.93	0.005
Anxiety								
	HAMA		STAI		HAMA		STAI	
Screening								
–day 10	4.9 ± 2.00	0.037	1.4 ± 1.71	0.435	6.1 ± 2.38	0.031	4.8 ± 2.63	0.101
–day 20	7.6 ± 2.59	0.017	1.3 ± 2.37	0.597	12.6 ± 3.04	0.003	7.2 ± 2.30	0.012
–day 30	9.1 ± 3.35	0.024	3.8 ± 2.57	0.174	16.5 ± 3.23	0.001	13.3 ± 3.17	0.002
Clinical global impression								
	CGI				CGI			
Screening								
–day 10	0.2 ± 0.20				0.5 ± 0.17			
–day 20	0.5 ± 0.34				1.2 ± 0.29			
–day 30	0.9 ± 0.31				1.9 ± 0.23			

suicidality, at day 20 in one case. These four cases were substituted and were not included in the analyses. They are described below and the impact of their exclusion upon the overall study outcome is discussed separately.

Fig. 3 illustrates the changes of the mean HAMD values over time and demonstrates that cessation of drug treatment resulted in a worsening of depressive symptomatology in 12 patients (six patients in each panel). These changes were independent from treatment outcome because worsening occurred among three nonresponders in panel 1 and three nonresponders in panel 2 (see Table 1). Similar trends emerged from analysis of the depression self-rating scores (see Table 2).

When anxiety symptoms were analysed separately significant improvements according to HAMA scores were again observed (Fig. 3). As summarized in Table 2, HAMA and STAI scores dropped in both panels, but these changes were more pronounced in panel 2, where following cessation of treatment,

anxiety symptoms also worsened. When comparing depression and anxiety ratings completed by the study clinicians (HAMD and HAMA) and by the patients (BDI and STAI), we found that at the time point of screening only depression-associated (HAMD and BDI) and anxiety-associated (HAMA and STAI) ratings seemed to be significantly correlated ($p < 0.05$), while construct-divergent correlations (HAMD/STAI or HAMA/BDI) were absent (see Table 3). These insignificant correlations reached significance ($p < 0.05$) at day 30, which is consistent with the treatment-related beneficial effects on both anxiety-related and depression-related symptoms. The differences of clinical

Table 3

Correlations of screening (baseline) anxiety and depression-related rating-scale scores according to Kendall's tau

	BDI	STAI
HAMD	0.34 ($p = 0.042$)	0.29 ($p = 0.083$)
HAMA	0.262 ($p = 0.117$)	0.391 ($p = 0.019$)

cian-rating scores compared with baseline (screening), started to reach the level of significance at day 10 (HAMD, HAMA) in both panels, while patient-rating scores improved significantly only in panel 2 at day 30 (BDI) and day 20 (STAI), indicating a faster decrease in panel 2 (see Table 2).

CGI scores also dropped significantly and here again the reduction of scores relative to the screening day was enhanced in panel 2, where significant improvements began to be noted at day 10. Similar effects emerged in panel 1, however, only at the end of the 30-day study (see Table 2).

3.5. Prediction of response

UFC values, plasma ACTH and cortisol concentrations at screening or during the study were not found to correlate with any measure of treatment outcome. However, the ACTH response measured at the first and second CRH test (days 9 and 19) was correlated with the HAMD and BDI response at the end of the study (see Table 4). At the end of the trial (day 29), when 13 patients had responded, such correlations were no longer found. Those patients who had a lower CRH-elicited ACTH response at the beginning of the study were more likely responders according to the HAMD or BDI criterion at the end of the study.

3.6. Drop-out cases

In order to test whether the differences observed between both panels (suggesting that the higher dose in panel 2 was superior to the lower dose in panel 1) were merely due to exclusion of four potentially nonresponding drop-out cases, we analysed the effect of these four drop-out cases according to an intention-to-treat analysis. This analysis used a last visit carry over approach, submitting that the data at the time point of study discharge would not have changed if the patients had been maintained. According to this conservative approach, the superiority of reductions in depression and anxiety severity in panel 2 is still present according to both clinicians' and patients' ratings, although the effects were less marked (see legend Table 2).

Table 4
Correlations (Spearman Rho) of Δ AUC values of CRH-stimulated net ACTH output with HAMD and BDI response scores at the end of the study

Δ AUC	HAMD response		BDI response	
	Spearman Rho	<i>p</i>	Spearman Rho	<i>p</i>
Post CRH-pre CRH				
ACTH: day 9	-0.46	0.04	-0.43	0.06
ACTH: day 19	-0.60	0.007	-0.62	0.005
ACTH: day 29	-0.19	0.42	-0.30	0.20

The UFC values also decreased moderately when drop-out cases were included and this reduction in UFC values was more pronounced in panel 2. The plasma ACTH and cortisol levels which were found to be unaffected by R121919 among those completing the study in both panels remained unaffected when the four drop-out cases were included into the analyses. In addition, inclusion of drop-out cases did not invalidate the conclusion that treatment with R121919 failed to impair HPA responsiveness to CRH stimulation. The observation that blunted ACTH responses to CRH stimulation was predictive for a favorable response to treatment with R121919 and that high ACTH release indicates a less-favorable outcome was also confirmed following inclusion of the four drop-out cases.

4. Discussion

The main purpose of this study was to test whether a drug that antagonizes CRH₁ receptors is safe and well tolerated when administered to patients suffering from major depression. The clinical monitoring of laboratory tests, including clinical chemistry, ECG and EEG, proved that administration of R121919 was safe under the dose range and during the time period tested. Moreover, the drug was very well tolerated as none of the patients reported any subjective adverse effects. The effect of R121919 on endocrine regulation was of particular interest, since in rodents blockade of CRH₁ receptors had reduced their corticotrophic responsiveness to CRH. This could be a problem whenever an acute stress-induced increase of ACTH and cortisol is needed as part of an individual's defense mechanism, e.g. in the case of inflammation (Webster et al., 1996). Acute hormonal stress response is mediated by the CRH produced and released from the hypothalamus and acting at corticotrophs. Because cognitive or physical stressors would have been inappropriate under the clinical condition, intravenous CRH stimulations were repeatedly administered at escalating dosages of R121919 and, as illustrated in Fig. 2, the CRH-elicited ACTH secretions were indistinguishable across all dosages ranging from 5 to 80 mg, strongly suggesting that a complete blockade of the peripheral stress hormone system was unlikely. This interpretation is supported by the comparisons of UFC values, which found that this measure of overall adrenocortical secretory activity decreased only to a minor extent during treatment in both panels. This change can be attributed to the clinical improvement known to be associated with decreased HPA drive rather than to drug-induced HPA impairment (Holsboer and Barden, 1996). In fact, the UFC values remained in the normal range at any time point tested. Similarly, no specific effects of R121919 upon the cir-

cadian rhythms of plasma ACTH and cortisol secretion was noted, rejecting the possibility that partial blockade of CRH₁ receptors may interfere with circadian changes of HPA activity. In accordance with UFC values which only slightly decreased during treatment with R121919 in both panels, the mean plasma cortisol levels pooled over all five samplings tended to decrease, which is in accord with well-established effects of antidepressants on HPA activity in major depression (Holsboer and Barden, 1996). The lack of suppression of ACTH and cortisol by a CRH₁ receptor antagonist is consistent with the view that basal corticotrophic activity is independent from CRH. This is supported in preclinical studies where mice lacking functional CRH₁ receptors have unchanged plasma ACTH and corticosterone levels at baseline (Timpl et al., 1998). When confronted with a physical stressor, however, or when exposed to CRH, the corticotrophic cells of mouse mutants without CRH₁ receptors and of rats treated with high dosages of R121919 have suppressed plasma ACTH and corticosterone secretions which contrasts with the findings reported here (Timpl et al., 1998; Keck et al., unpublished observations). Nonhuman primates, however, were found to have CRH₂ receptors in the pituitary (Sánchez et al., 1999), and if this is also true in humans, the releasability of ACTH following a CRH infusion suggests that in the case of partial or even complete blockade of CRH₁ receptors appropriate ACTH release may be achieved through corticotrophic CRH₂ receptors. Because decrease or absence of CRH₁ receptors in heterozygous or homozygous null-mutant mice is associated with increased production and release of vasopressin, an amplification of CRH effects upon ACTH release may occur (Müller et al., unpublished observation; de Goeij et al., 1991; Wotjak et al., 1996). Alternatively, the blockade of corticotrophic CRH₁ receptors by R121919 under the current study conditions could have been incomplete, leaving sufficient CRH₁ receptors available for adequate CRH-elicited hormonal effects. While this possibility can not be completely rejected it seems unlikely to be the case because the CRH-stimulated ACTH release remained unchanged over a dose range from 5 to 80 mg of R121919.

We were interested as to whether neuroendocrine laboratory markers would predict the response of depressive symptomatology to R121919. Theoretically, if CRH hypersecretion would constitute a phenomenon that is present at all levels of CRH regulation and signaling, one would predict that the patients with peripheral indicators for HPA overactivity would be more likely to respond to a CRH-receptor antagonist. In the current study, baseline HPA measures (UFC and plasma ACTH and cortisol levels) at screening or during drug treatment were not predictive of clinical outcome. Interest-

ingly, CRH-stimulated ACTH measured after initiation of treatment with R121919 corresponded with the decrease in the HAMD score at the end of trial. One attractive explanation would be that increased CRH release from the limbic brain decreased the number of CRH receptors at the anterior pituitary, which results in the decreased responsiveness observed when stimulated by exogenous CRH. Thus, CRH hypersecreting patients as identified by blunted ACTH response to a CRH challenge, would be expected to more likely benefit from a CRH-receptor-blocking drug than patients with normal CRH secretory activity, having normal ACTH responses in CRH tests. This interpretation, however, is hampered by the differences in CRH and CRH-receptor regulation throughout the brain. While the CRH synthesis is suppressed at the level of the hypothalamus by glucocorticoids, either indirectly through protein-protein interactions between activated glucocorticoid receptors and transcription factors or directly through negative response elements in the CRH gene promoter (Malkoski and Dorin, 1999), expression of CRH can also be enhanced by glucocorticoids in other brain areas (Schulkin et al., 1998). In the amygdala, or those hypothalamic nuclei which project to the spinal cord, evidence has been provided that CRH expression is not suppressed but rather increased by glucocorticoids (Swanson and Simmons, 1989; Schulkin et al., 1998). Thus, CRH may be indirectly enhanced in the amygdala and cerebrospinal fluid by hypercortisolism originating from those hypothalamic nuclei projecting to the median eminence. It is likely that other regulatory elements such as CRH-binding protein (Potter et al., 1994) and as yet to be identified CRH receptors, are also participating in CRH effects at various neural circuits in the CNS. These complex interactions indicate that a peripheral HPA measure (UFC or CRH test results) is not necessarily reflective of the level of CRH₁-receptor activation in those brain areas implicated in the neuropathology of depression.

In this study, a number of psychopathometric scales were also applied which collectively showed significant reductions in the severity of depression and anxiety. Because of the open-label nature of the study any conclusions related to antidepressant effects of R121919 are limited. We can not rule out the possibility that nonspecific, placebo-like effects and clinician biases might have accounted for the changes in depression severity observed in the patients. Several observations support the notion that the data collected in this study give reason to further explore the potential of CRH-receptor antagonists as psychotropic drugs in controlled studies:

1. The group in panel 2 receiving higher drug dosages had a better overall response than patients receiving the lower dose regimen. This dose/response relationship speaks against a placebo-like effect.
2. After discontinuation of the drug a worsening of symptomatology occurred, which is an unlikely event under clinical routine ('wash-out') conditions and favors the possibility of a specific drug effect. This view is supported by the observation that not only patients who responded favorably, but also those with poor or absent treatment effects showed worsening of depressive psychopathology (HAMD scores) when discontinued from R121919 (see Fig. 3)
3. The possibility of clinician's biases as a major confound also seems unlikely because results from the patient-rated inventories (BDI and STAI) matched those of the clinician-rated instruments (HAMD, HAMA and CGI).

At the time of screening, when patients were examined prior to treatment there were only correlations between depression-related instruments (HAMD and BDI) and between anxiety-related instruments (HAMA and STAI). Construct-divergent correlations, i.e. between HAMD and STAI or HAMA and BDI were absent at screening. However, at the end of the study all rating scores, especially their changes between screening and endpoint were strongly correlated, which agrees with a positive overall effect that can not be primarily attributed to a sole anxiolytic effect. If the latter were the case, the patient- and clinician-rated depression scores would have been correlated with the corresponding anxiety scores at enrollment.

In this open-label study we have found significant reductions in patient- and clinician-rated depression and anxiety scores. Comparing the degree of clinical improvement over subsequent clinical ratings it becomes apparent that these changes tended to be earlier and more pronounced among those patients who entered panel 2, receiving higher dosages. Because of this dose-response effect the high correlations between patients' and clinicians' rated changes in symptom scores, and the outcome of independent worsening of psychopathology after drug discontinuation, we believe that the observations reported here can not be attributed just to methodological issues. Of course, we are aware that the findings reported here are preliminary and that the purpose and design of the study allowed only descriptive analysis.

The clinically observed beneficial effects on depressive symptoms, as well as the absence of clinically relevant adverse effects, particularly interference with neuroendocrine regulation, justify validation of the postulated efficacy of R121919 in controlled trials. Another suggestion from the current study is the need for clinical tests that help to identify those patients

which are likely to respond to R121919 and other CRH₁-receptor antagonists in the future. It is important to note that the activity of CRH neurons does not change in a uniform way in the brain, and increased CRH signaling through CRH₁ receptors is not necessarily manifested by increased hypothalamic CRH secretion into portal vessels. Thus, measuring ACTH and cortisol in the periphery might only poorly predict clinical response to a CRH₁-receptor antagonist.

Another issue emerging from this and other studies trying to elaborate the therapeutic potential of drugs that work through new nonconventional mechanisms relates to diagnostic boundaries. CRH-receptor antagonists work through a mechanism that prevents the consequences of hyperexposure of neural substrates to CRH (Holsboer, 1999). The resulting excessive CRH-receptor signaling may have many reasons ranging from genetically impaired corticosteroid-receptor signaling that leads to impaired negative feedback upon CRH secretion to the sequelae of severe trauma, particularly during early childhood. The consequence of such genetic and/or acquired impairments of HPA regulation renders an individual likely to develop a psychiatric disease, particularly under conditions of stressful events. Thus, future studies exploring the therapeutic potential of CRH-receptor antagonists will encompass not only depression but also anxiety disorders, stress-related sleep disorders, anorexia, stress-induced psychoses and withdrawal from substance abuse.

Acknowledgements

Part of this study was funded by Janssen Research Foundation (GER1).

References

- Chalmers DT, Lovenberg TW, Grigoriadis DE, Behan DP, De Souza EB. Corticotropin-releasing factor receptors: from molecular biology to drug design. *Trends Pharmacol Sci* 1996;17:166–72.
- Chen R, Lewis KA, Perrin MH, Vale WW. Expression cloning of a human corticotropin-releasing factor receptor. *Proc Natl Acad Sci USA* 1993;90:8967–71.
- Coplan JD, Andrews MW, Rosenblum LA, Owens MJ, Friedman S, Gorman JM, Nemeroff CB. Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult non-human primates exposed to early-life stressors: implications for the pathophysiology of mood and anxiety disorders. *Proc Natl Acad Sci USA* 1996;93:1619–23.
- Davis M. The role of the amygdala in fear and anxiety. *Ann Rev Neurosci* 1992;15:353–75.
- De Goeij DCE, Kvetnansky R, Whitnall MH, Jezova D, Berkenbosch F, Tilders FJH. Repeated stress-induced activation of corticotropin-releasing factor neurons enhances vasopressin stores and colocalization with corticotropin-releasing factor in the median eminence of rats. *Neuroendocrinology* 1991;53:150–9.

- Gold PW, Chrousos G, Kellner C, Post R, Roy A, Augerinos P, Schulte H, Oldfield E, Loriaux DL. Psychiatric implications of basic and clinical studies with corticotropin-releasing factor. *Am J Psychiatry* 1984;141:619–27.
- Grigoriadis DE, De Souza EB. Small molecule CRF₁ receptor antagonists: characterization and clinical application. In: *Neuroendocrine workshop on stress sponsored by the Am. Neuroendocrine Soc.*; June 21–23, 1998, New Orleans, LA, 1998. p. 36.
- Heinrichs SC, Lapansky J, Lovenberg TW, De Souza EB, Chalmers DT. Corticotropin-releasing factor CRF₁, but not CRF₂ receptors mediate anxiogenic-like behavior. *Regul Peptides* 1997;71:15–21.
- Holsboer F. Neuroendocrinology of mood disorders. In: Bloom FE, Kupfer DJ, editors. *Psychopharmacology: The fourth generation of progress*. New York: Raven Press, 1995. p. 957–69.
- Holsboer F. The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety. *J Psychiat Res* 1999;33:181–214.
- Holsboer F, Barden N. Antidepressants and HPA regulation. *Endocrine Rev* 1996;17:187–205.
- Holsboer F, von Bardeleben U, Gerken A, Stalla GK, Müller OA. Blunted corticotropin and normal cortisol response to human corticotropin-releasing factor in depression. *N Engl J Med* 1984;311:1127.
- Holsboer F, Gerken A, von Bardeleben U, Grimm W, Beyer H, Müller OA, Stalla GK. Human corticotropin-releasing hormone in depression. *Biol Psychiatry* 1986;21:601–11.
- Holsboer F, Spengler D, Heuser I. The role of corticotropin-releasing hormone in the pathogenesis of Cushing's disease, anorexia nervosa, alcoholism, affective disorders and dementia. *Prog Brain Res* 1992;93:385–417.
- Kalin NH. Biological effects of corticotropin-releasing hormone administered to rhesus monkeys. *Fed Proc* 1985;44:249–53.
- Keck FE, Holsboer F (2000): Hyperactivity of CRH neuronal circuits as a target for therapeutic interventions in affective disorders. *Peptides* (In press).
- Liebsch G, Landgraf R, Gerstberger R, Probst JC, Wotjak CT, Engelmann M, Holsboer F, Montkowski A. Chronic infusion of a CRH₁ receptor antisense oligodeoxynucleotide into the central nucleus of the amygdala reduced anxiety-related behavior in socially defeated rats. *Regul Peptides* 1995;59:220–39.
- Liebsch G, Montkowski A, Holsboer F, Landgraf R. Behavioural profiles of two Wistar rat lines selectively bred for high or low anxiety-related behaviour. *Behav Brain Res* 1998;94:301–10.
- Liebsch G, Landgraf R, Engelmann M, Lörscher P, Holsboer F. Differential behavioural effects of chronic infusion of CRH₁ and CRH₂ receptor antisense oligonucleotides into the rat brain. *J Psychiat Res* 1999;33:153–63.
- Malkoski SP, Dorin RI. Composite glucocorticoid regulation at a functionally defined negative glucocorticoid response element of the human corticotropin-releasing hormone gene. *Mol Endocrinol* 1999;13:1629–44.
- Nemeroff CB, Widerlov E, Bissette G, Walleus H, Karlsson E, Eklund K, Kilts DC, Loosen PT, Vale W. Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* 1984;226:1342–4.
- Nemeroff CB, Owens MJ, Bissette G, Andorn AC, Stanley M. Reduced corticotropin-releasing factor binding sites in the frontal cortex of suicide victims. *Arch Gen Psychiatry* 1988;45:577–9.
- Owens MJ, Nemeroff CB. The physiology and pharmacology of corticotropin-releasing factor. *Pharmacol Rev* 1991;43:425–73.
- Plotsky PM. Pathways to the secretion of adrenocorticotropin: a view from the portal. *J Neuroendocrinol* 1991;3:1–9.
- Potter E, Sutton S, Conaldson C, Chen R, Perrin M, Lewis K, Sawchenko PE, Vale W. Distribution of corticotropin-releasing factor receptor mRNA expression in the rat brain and pituitary. *Proc Natl Acad Sci USA* 1994;91:8777–81.
- Raadsheer FC, Hoogendijk WJG, Stam FC, Tilders FJH, Swaab DF. Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. *Neuroendocrinology* 1994;60:433–6.
- Sánchez MM, Young LJ, Plotsky PM, Insel TR. Autoradiographic and in situ hybridization localization of corticotropin-releasing factor 1 and 2 receptors in nonhuman primate brain. *J Comp Neurol* 1999;408:365–77.
- Schulkin J, Gold PW, McEwen BS. Induction of corticotropin-releasing hormone gene expression by glucocorticoids: implication for understanding the states of fear and anxiety and allostatic load. *Psychoneuroendocrinology* 1998;23:219–43.
- Schulz DW, Mansbach RS, Sprouse J, Braselton JP, Collins J, Corman M, Dunaikis A, Faraci S, Schmidt AW, Seeger T, Seymour P, Tingley 3rd FD, Winston EN, Chen YL, Heym J. CP-154,526: A potent and selective nonpeptide antagonist of corticotropin-releasing factor receptors. *Proc Natl Acad Sci USA* 1996;93:10477–82.
- Shaham Y, Erb S, Leung S, Buczek Y, Stewart J. CP-15,526, a selective, nonpeptide antagonist of the corticotropin-releasing factor₁ receptor attenuates stress-induced relapse to drug seeking in cocaine- and heroine-trained rats. *Psychopharmacology* 1998;137:184–90.
- Skutella T, Probst JC, Criswell H, Moy C, Breese G, Jirikowski GF, Holsboer F. Antisense oligodeoxynucleotide complementary to corticotropin-releasing hormone mRNA reduces anxiety in shuttlebox performance. *Neuroreport* 1994;5:2181–5.
- Skutella T, Probst JC, Renner U, Holsboer F, Behl C. Corticotropin-releasing hormone receptor (type 1) antisense targeting reduces anxiety. *Neuroscience* 1998;85:795–805.
- Smith GW, Aubry J-M, Dellu F, Contarino A, Bilezikjian IM, Gold LH, Hauser C, Bentley CA, Sawchenko PE, Koob GF, Vale W, Lee K-F. Corticotropin-releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. *Neuron* 1998;20:1093–102.
- Steckler T, Holsboer F. Corticotropin-releasing hormone receptor subtypes and emotion. *Biological Psychiatry* 1999;46:1480–508.
- Stenzel-Poore MP, Heinrichs SC, Rivest S, Koob GF, Vale WW. Overproduction of corticotropin-releasing factor in transgenic mice: a genetic model of anxiogenic behavior. *J Neurosci* 1994;14:2579–84.
- Swanson LW, Simmons DM. Differential steroid hormone and neural influences on peptide mRNA levels in CRH cells of the paraventricular nucleus: a hybridization histochemical study in the rat. *J Comp Neurol* 1989;285:415–35.
- Timpl P, Spanagel R, Sillaber I, Kresse A, Reul JMH, Stalla GK, Blanquet V, Steckler T, Holsboer F, Wurst W. Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. *Nature Genet* 1998;19:162–6.
- Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β -endorphin. *Science* 1981;213:1394–7.
- Valentino RJ, Foote SL, Page ME. The locus coeruleus as a site for integrating corticotropin-releasing factor and noradrenergic mediation of stress responses. *Ann NY Acad Sci* 1993;697:173–88.
- Webster EL, Lewis DB, Torpy DJ, Zachman EK, Rice KC, Chrousos GP. In vivo and in vitro characterization of antalarmin, a non-peptide corticotropin-releasing hormone (CRH) receptor antagonist: suppression of pituitary ACTH release and peripheral inflammation. *Endocrinology* 1996;137:5747–50.
- Wotjak CT, Kubota M, Liebsch G, Montkowski A, Holsboer F, Neumann I, Landgraf R. Release of vasopressin within the rat paraventricular nucleus in response to emotional stress: a novel mechanism of regulating adrenocorticotrophic hormone secretion? *J Neuroscience* 1996;16:7725–32.

Robertson, Ed.

oda, K. Koshiya, M.

acol.Exp.Ther., 283,

, P.A. Sargent, C.J.
R5 (1996).

arez, M. Marien, and

and J.L. Moreau,

rn, and U. Widmer,

nd J. Wichmann,

ot, Ed., Elsevier,

it, and U. Widmer,

Guardiola-Lemaitre,

I.L. Grassam, P.M.

wman, G. Riley, C.

, Jr., Drug News &

in, Br.J.Pharmacol.,

owski, E. Ber, G.G.
ill, N.M.J. Rupniak,
d.Chem., 41, 4607

amek, S.A. Reines,
oss, C.J. Swain, T.
, S. Sadowski, A.R.
d N.M.J. Rupniak,

joer, A. Pasini, and

ahir, and D. Beilelli,

in, C.L. Kimbrough,
N. Gee, and M.B.

n, R.M. Woodward,

Q, 119 (1996).

Chapter 2. Recent Progress in Corticotropin-Releasing Factor Receptor Agents

James R. McCarthy*, Stephen C. Heinrichs† and Dimitri E. Grigoriadis
Neurocrine Biosciences Inc., San Diego, CA 92121-1102

*Lilly Corporate Center, Eli Lilly and Company, Indianapolis, IN 46285
†Psychology Dept., Boston College, Chestnut Hill, MA 02467

Introduction - Corticotropin-releasing factor (CRF) is a neurohormone which appears necessary and sufficient for the organism to mount functional, physiological and endocrine responses to stressors (1). Factors which mobilize brain CRF systems appear to have one feature in common, -the ability to disturb homeostasis. For example, demands on the organism may be induced either internally or externally by exposure to physical trauma, infection or social conflict. Coping responses to such deflections in steady state which include sympathetic nervous system activation, promotion of negative energy balance and augmentation of vigilance and emotionality appear to be CRF-dependent. Not surprisingly, many human psychopathologies which include hyperexcitability or anxiety-like components are hypothesized to depend either causally or symptomatically on overactivation of CRF in brain. This chapter will document recent progress towards the goal of assigning functional significance to endogenous CRF circuits in brain, and examine the state of the art regarding the growing array of pharmacological tools, including the most recently published small-molecule ligands, which are available for further probing the physiological significance of CRF system activation.

FUNCTIONAL SIGNIFICANCE OF CRF RECEPTORS AND CRF-BINDING PROTEIN

CRF Receptors - CRF receptors belong to the recently described family of "gut-brain" neuropeptide receptors. Other typical members of this family include receptors for calcitonin, vasoactive intestinal peptide, parathyroid hormone, secretin, pituitary adenylate cyclase-activating peptide, glucagon and growth hormone-releasing factor. All of these receptors possess seven putative transmembrane domains and are positively coupled to adenylyl cyclase.

The CRF₁ receptor was first cloned from several species including human (2, 3), mouse (3) and rat (4, 5). Species homologs are 98% identical over their full length of 415 amino acids. In general, the CRF₁ receptor is approximately 30% identical to all other members of the neuropeptide receptor family. Characteristic of most G-protein coupled receptors, the CRF₁ receptor has putative N-linked glycosylation sites on the N-terminal extracellular domain. There are five predicted sites on CRF₁, substantiating the glycosylation profiles determined by chemical affinity cross-linking studies (6). In addition, there are potential protein kinase C phosphorylation sites in the first and second intracellular loops and in the C-terminal tail, as well as casein kinase II and protein kinase A phosphorylation sites in the third intracellular loop (2).

There are currently three known forms of the CRF₂ receptor: CRF_{2a}, CRF_{2b} and CRF_{2c}. The CRF_{2a} receptor, which was originally described by Lovenberg et al. (7), is a 411 amino acid protein with approximately 71% identity to the CRF₁ receptor. The

CRF_{2B} receptor, which has been cloned from both rat (7) and mouse (8, 9), is 431 amino acids in length and differs from CRF_{2A} in that the first 34 amino acids in the N-terminal extracellular domain are replaced by 54 different amino acids. A third splice variant, the CRF_{2C} receptor, has recently been identified in human brain (10). This splice variant uses yet a different 5' alternative exon for its amino terminus and replaces the first 34 amino acid sequence of the CRF_{2A} receptor with a unique 20 amino acid sequence. RT-PCR analysis of human brain mRNA demonstrated expression in amygdala and hippocampus while southern analysis of rat genomic DNA yielded negative results, suggesting that this subtype does not exist in rat. All splice variants of the CRF₂ receptor have potential N-glycosylation and phosphorylation sites, which are analogous to those found in CRF₁ receptors. It is interesting to note that there are very large regions of amino acid identity between CRF₁ and CRF₂ receptors, particularly between transmembrane domain five and transmembrane domain six. This similarity argues strongly for conservation of biochemical function since it is this region which is thought to be the primary site of G-protein coupling and signal transduction.

CRF Binding-Protein (CRF-BP) - Plasma CRF is substantially elevated during the third trimester of human pregnancy and this process is likely to participate in a cascade of events which eventually leads to parturition (11). It was subsequently demonstrated that the majority of this late gestational maternal plasma CRF is bound to a high affinity CRF-binding protein (CRF-BP) which neutralizes the ability of CRF to release adrenocorticotrophic hormone (ACTH). Thus, the levels of CRF-BP in the maternal plasma determine the amount of 'free' CRF that is available to interact with pituitary CRF receptors and thereby modulate the activity of the pituitary-adrenocortical axis during late human pregnancy. The predominant tissues expressing CRF-BP in all species are the brain and the pituitary gland where the protein is hypothesized to regulate CRF actions (12). With respect to the CNS and the role of CRF-BP, it has recently been demonstrated that CRF-BP is expressed in various areas of brain including the cerebral cortex, amygdala, hippocampus, hypothalamus as well as sensory relays associated with the auditory, olfactory, vestibular and trigeminal systems. Of note, there are brain areas that are enriched with CRF and CRF-BP but have only low densities of receptors and conversely, other brain areas which are enriched with receptors and devoid of CRF-BP (13). Thus, the differential distribution of brain CRF-BP and CRF receptors presents multiple distinct sites of interaction with CRF for potential exploitation in the treatment of central deficits in CRF neurotransmitter function.

CRF Mutant Mouse Models - The profusion of CRF peptide, CRF post-synaptic receptor and CRF binding-protein transgenic and knockout mouse models reported over the past year allows for critical analysis of hypotheses relating pituitary-adrenocortical axis tone and brain CRF system activation in animal models to a variety of clinical psychopathologies. One phenotype common to all of the CRF mutants is altered tone/activity of the pituitary-adrenocortical axis. CRF overexpressor (transgenic) mice exhibit an overabundance of CRF in brain as well as 5-10 fold elevations in plasma levels of ACTH and corticosterone (14). CRF-knockout mice which are CRF deficient lose normal circadian variations in plasma ACTH and corticosterone which are restored by constant infusion of exogenous CRF (15). CRF₁

receptor knock attenuated AC1 (16). CRF binds stimulated plas consequences in expression accommodate at different se adrenocortical

The putative CRF₁ receptor I mice. The phe CRF systems c under- or over overexpressor flight response, knockdown, CR like attributes (1 CRF-BP which c like properties in knockout mice v emotionality in t mediating huma

CRF Gene Ki oligonucleotide translational arr expected to be administration o directed against vehicle-treated the antisense-t procedure (20). behaviors in p. attenuates the Consistent with nucleus of the socially defeated receptors did n suggesting that related behavior

Human Stress-A of CRF has be consumption, di behavioral char psychiatric diso

se (8, 9), is 431
acids in the N-
s. A third splice
brain (10). This
o terminus and
ith a unique 20
demonstrated
of rat genomic
exist in rat. All
cosylation and
receptors. It is
identity between
lomain five and
conservation of
primary site of G-

d during the third
in a cascade of
ly demonstrated
ound to a high
f CRF to release
in the maternal
act with pituitary
renocortical axis
g CRF-BP in all
hypothesized to
f CRF-BP, it has
areas of brain
mus as well as
and trigeminal
and CRF-BP but
areas which are
ential distribution
of interaction with
deficits in CRF

RF post-synaptic
models reported
relating pituitary-
models to a variety
CRF mutants is
F overexpressor
well as 5-10 fold
F-knockout mice
asma ACTH and
CRF (15). CRF₁

receptor knockout mice display low basal levels of plasma corticosterone and attenuated ACTH and corticosterone stimulation in response to a restraint stressor (16). CRF binding protein transgenic mice which exhibit a normal pattern of basal and stimulated plasma ACTH and corticosterone levels appear to counterbalance the consequences of CRF binding protein enrichment via a compensatory 82% increase in expression of CRF (17). It is important to note that while the CRF mutants accommodate their genetic re-programming by adaptation of the endocrine stress axis at different set points, each mutant retains the ability to activate the pituitary-adrenocortical axis, albeit in diminished or altered fashion, in response to stress.

The putative role of brain CRF in affective disorders has been examined using CRF₁ receptor knockout mice and CRF binding-protein overexpressing and knockout mice. The phenotype of CRF mutants with under- or over-activation of endogenous CRF systems can now be compared to the expected consequences of stress-axis under- or overactivation predicted by clinical findings (18). For instance, CRF overexpressor mice exhibit an anxiogenic-like phenotype characteristic of a fight or flight response, whereas deactivation of brain CRF systems via CRF neuropeptide knockdown, CRF₁ receptor knockdown or knockout evokes complementary, anxiolytic-like attributes (19). Bionutralization of CRF by an indirect means, overexpression of CRF-BP which complexes and neutralizes endogenous CRF, also produces anxiolytic-like properties *in vivo*. Elucidation of affective phenotypes for CRF and CRF₂ receptor knockout mice will soon be possible. The positive correlation between CRF levels and emotionality in these animal models is in keeping with the hypothesized role of CRF in mediating human affective diseases.

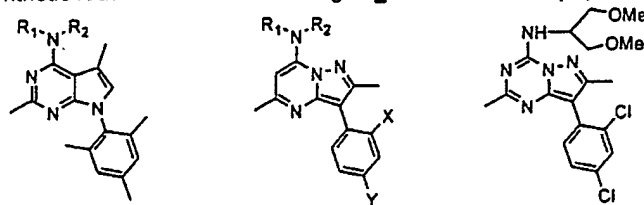
CRF Gene Knockdown - Gene knockdown, also referred to as antisense oligonucleotide targeting, suppresses expression of a particular gene product via translational arrest. Thus, the *in vivo* consequences of gene knockdown would be expected to be consistent with the pattern of functional effects of gene deletion or administration of competitive receptor antagonists. Treatment of rats with an antisense directed against CRF was shown to produce anxiolytic-like effects. Compared to vehicle-treated animals, lower ACTH and corticosterone levels were also observed in the antisense-treated animals following exposure to a shuttle-box avoidance procedure (20). Recent antisense studies focused on CRF₁ and anxiety-related behaviors in particular have demonstrated that treatment with CRF₁ antisense attenuates the anxiogenic-like effects induced by central administration of CRF (21). Consistent with this finding, chronic infusion of a CRF₁ antisense into the central nucleus of the amygdala was also reported to reduce anxiety-related behaviors in socially defeated rats (22). In contrast, treatment with antisense directed against CRF₂ receptors did not affect performance in the defensive withdrawal paradigm, thus suggesting that CRF₂ may not participate in the neural pathways mediating anxiety-related behaviors (23).

Human Stress-Axis Pathophysiology - In rodent animal models, central administration of CRF has been demonstrated to produce anxiety-like effects, decreased food consumption, diminished sexual behavior and altered sleep patterns (24). These behavioral changes closely parallel signs and symptoms observed in human psychiatric disorders including major depression, anxiety disorders and anorexia

nervosa, thus suggesting a role for CRF in the pathophysiology of mental illness. Accordingly, CRF hypersecretion has been detected in a large portion of individuals diagnosed with major depression (18). In depressed patients, elevated cortisol levels and a blunted ACTH response to CRF administration were observed, thus supporting the notion that the hypercortisolism observed in major depression is due to abnormal CRF secretion in the central nervous system. Noteworthy, treatment of depressed patients with antidepressant drugs or electroconvulsive therapy resulted in a decrease of the altered levels of CRF observed before the antidepressant treatment as well as in improvements in the clinical conditions of these patients. This observation strengthens the hypothesis that overactivation in brain CRF pathways may underlie certain features of the symptomatology seen in affective disorders, in which case CRF receptor antagonists would be expected to have a therapeutic benefit. In complementary fashion, CRF receptor agonist administration could conceivably counteract the consequences of diminished activation of brain CRF circuits (25).

CRF RECEPTOR ANTAGONISTS, AGONISTS AND CRF-BP LIGAND INHIBITORS

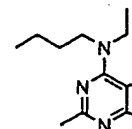
Non-peptide CRF₁ receptor antagonists that can selectively block the CRF₁ receptor subtype inhibit CRF-stimulation of cAMP or CRF-stimulated ACTH release from cultured rat anterior pituitary cells (26). Furthermore, when administered peripherally, these compounds compete for *ex vivo* [¹²⁵I]sauvagine binding to CRF₁ receptors in brain sections demonstrating their ability to cross the blood-brain-barrier. Peripheral administration of these compounds attenuates stress-induced elevations in plasma ACTH levels demonstrating that CRF₁ receptors can be blocked in the periphery. Furthermore, peripherally administered CRF₁ receptor antagonists have also been demonstrated to inhibit CRF-induced seizure activity (27) suggesting that non-peptide CRF₁ receptor antagonists, when administered systemically, also specifically block central CRF₁ receptors. Extensive evidence validates the ability of CP-154,526 (**1**) to competitively antagonize CRF₁ receptors. This compound attenuates fear-potentiated startle after oral administration in rats (28) and in the same study the oral bioavailability of **1** was estimated to be 37%. Comparison of the inhibition of binding of 70 pM [¹²⁵I]Tyr⁰-o-CRF to rat brain by the monobutyl analog (**2**) (IC₅₀ = 110 nM) and monoethyl analog (**3**) (IC₅₀ = 620 nM) of **1** (IC₅₀ = 5.5 nM) was made. A synthetic route to a ditritiated analog of **1** is now available (28).



- 1:** R₁ = n-Bu, R₂ = Et **4:** R₁ = H, R₂ = CH(Et)₂, X = CH₃, Y = OCH₃
2: R₁ = n-Bu, R₂ = H **5:** R₁ = CH₂-c-Pr, R₂ = n-Pr, X = Y = Cl
3: R₁ = H, R₂ = Et **6:** R₁ = n-Pr, R₂ = CH₂CH₂OCH₃, X = Y = OCH₃

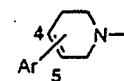
The efforts of several groups have focused on the synthesis of pyrazolo[1,5-a]pyrimidines as CRF receptor antagonists. After the publication of the first patent application (29), two additional world patent applications appeared (30, 31). The pharmacokinetics in dog of SP904 (**4**) (K_i = 1 nM) showed t_{1/2} (po) = 45 hours and oral

bioavailability of : potent analog, cyclopropylmethyl SAR study, NBI demonstrated effi 696, (**7**), (K_i = 1 pyrazolo[1,5-a]-s- t_{1/2} = 33.4 hours cardiovascular, C those effective in molecule improve



- 8:** R₁ = H
9: R₁ = CH₃
10: R₁ = CH₂-c-P

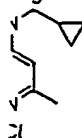
Certain N-7 imidazopyridine-2 small N-7 alkyl gr comparing **8** (K_i : the 8-oxo group : while the 1-dea; d]pyrimidine thio substituted pher antagonists. CR_A found to have (compounds to r activity amelioral aminopyrimidine- 41). DMP 695 (**1** receptor antagon



- 14:** Ar :
15: Ar :

While a wide molecule CRF₁ r which has receiv may correlate w increase expone possibly serve a CRF levels were that CRF recep hypothesis, infus

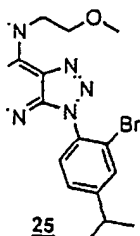
reported by the agonists.



1

it CRF receptor
AR study on the
5 nM) reported a

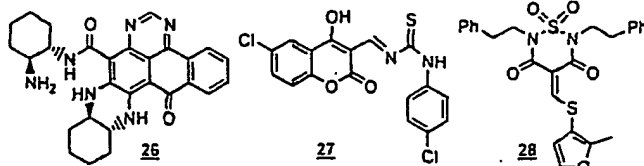
atic SAR studies
ilar to a model
s to rotation of
ods, X-ray and
ved the active
dditional anilino-
24) ($K_i = 32$ nM)
ral bioavailability
ours. However,
yclic triazolo[4,5-
udy, SC241 (25)
ntial therapeutic
d (51).



25

(26), the number
here is a scarcity
ve appeared that
eneric structures
le CRF receptor

labeled o-CRF
Compound 26
 R_1 /CRF $_2$ receptor
ation and CRF-
g an antagonist.
ber of different
00 nM), D_2 ($K_i =$
against the other



The first patent application for CRF binding protein ligand inhibitors claiming structure 27, as well as related compounds, has recently appeared (54). Rats were treated with 1 mg/kg (po) of 27 and were subjected to the Morris Water Maze test. A slight improved performance was reported over control rats after three days of acquisition training. In a second patent application (55), compound 28 and related structures, were claimed as CRF binding protein ligand inhibitors. No biological data appeared in the patent application.

CRF RECEPTOR AND CRF-BP SELECTIVE PHARMACOLOGICAL TOOLS

Construction of CRF Peptide Analogs – The native rat/human CRF(1-41) peptide affords a variety of opportunities to study CRF neurobiology by virtue of the fact that discrete and separate sequences of amino acids within the peptide chain subserve CRF receptor binding, receptor signaling and CRF-BP binding functions (56). For instance, the cyclization of shortened agonists of CRF at positions 30–33 to form a lactam ring (e.g. cyclo(30-33)[Ac-Leu⁸,D¹²Phe¹²,Nle²¹,Glu³⁰,Lys³³,Nle³⁸]hCRF(8-41) (or the equivalent positions 29-32 in sauvagine and urocortin) induces an α -helical constraint that stabilizes a bioactive conformation of the peptides. In addition, evidence was presented that the leucine/isoleucine residues at position 8 of CRF peptide agonists bears sole responsibility for activation of the receptor (57). Similarly, the tertiary structure of CRF necessary to bind its postsynaptic receptors is provided by the aminoterminal region of the peptide whereas amino acids near the carboxyterminus are necessary for binding CRF-BP (58). These inherent physicochemical properties allow endogenous CRF family agonists such as r/h CRF(1-41) and rat or human urocortin (1-40) to be modified forming peptides such as α -helical CRF(9-41) or d-Phe CRF(12-41) which are competitive receptor antagonists and peptides such as r/h CRF(6-33) which is a selective CRF-BP ligand inhibitor (Table 1). This same approach may also provide receptor selective agonist peptides (59) which can guide the discovery of small molecule agonists such as 18 and 19.

In Vivo Efficacy Profile of CRF Receptor and CRFBP Ligands – The differential binding profile for CRF family ligands exhibited in Table 1 is mirrored by distinct functional actions following administration in animal models. For example, the competitive CRF receptor antagonists, α -helical CRF (9-41) and d-Phe CRF (12-41) both block CRF $_1$ and CRF $_2$ receptors and yet are easily distinguished *in vivo* by the fact that α -helical CRF (9-41), but not d-Phe CRF (12-41), produces partial agonist actions (60) which may be due to affinity of α -helical CRF (9-41) for the CRF binding-protein. This supposition is supported by functionally selective *in vivo* effects of an indirect CRF agonist, the competitive CRF binding-protein ligand inhibitor peptide rat/human CRF (6-33), relative to the profile of a full post-synaptic CRF $_1$ /CRF $_2$ receptor agonist such as CRF itself (61). Similarly, the rank order in affinity of urocortin (CRF binding-protein > CRF $_2$ receptor > CRF $_1$ receptor) favors a functional dissociation from CRF itself which has a slightly different rank order profile (CRF binding protein > CRF $_1$ receptor > CRF $_2$ receptor). Consistent with this interpretation, the dissociation kinetics of CRF and urocortin from CRF binding-protein reveal an irreversible binding profile for urocortin (62) which could account, in part, for differential *in vivo* efficacy of urocortin versus CRF reported in some studies (63). Urocortin has also been examined in a variety of rodent behavioral and physiological assays of motor activity, exploratory inhibition, cardiovascular tone, malaise and food intake.

These paradigms were selected for their sensitivity to central nervous system and physiological activation states induced by exposure to environmental stressors and exogenous administration of CRF-like peptides such as CRF itself, sauvagine and urotensin. Anorexic actions of urocortin *in vivo* appear to be analogous to those of CRF, although urocortin has been reported to be the more potent of the two agonists in reducing food intake in some (63) but not all (64) studies. Thus, the precise neurobiological substrates for *in vivo* actions of CRF-family neuropeptides will likely follow extensive pharmacological studies employing CRF receptor subtype and CRF binding-protein selective ligands.

Table 1. Binding Profile of CRF Family Ligands

Ligand	CRF ₁	CRF ₂	CRF-BP
r/h CRF(1-41)	Yes	Yes	Yes
r/h urocortin (1-40)	Yes	Yes	Yes
r/h CRF(6-33)	No	No	Yes
Non-peptide CRF-BP	No	No	Yes
Ligand Inhibitor (27,28)			
o α -helical CRF(9-41)	Yes	Yes	Yes
r/h d-Phe CRF(12-41)	Yes	Yes	No
CP-154,526 (1)	Yes	No	No
Non-peptide CRF ₁ /CRF ₂	Yes	Yes	No
Receptor Antagonist (25)			

Summary - The pace of discovery for CRF receptor antagonists has substantially increased in the last two years as the interest in antagonists by the pharmaceutical industry has increased. As noted in the conclusions of the last report in this series (26), there are three key challenges in the area of CRF research. These include: 1) the discovery of CRF₂ subtype-selective agents to define the physiological roles of these sites; 2) the clinical evaluation of CRF₁-selective ligands and 3) the discovery of subtype-selective nonpeptide radioligands to better define the anatomical distribution of these receptors. Substantial progress has been made on all three fronts since there are now small molecule CRF₂ receptor antagonists, albeit with mixed CRF₁/CRF₂ activity, as well as results of ongoing clinical trials with selective non-peptide CRF₁ receptor antagonists that should be available in the near future.

References

1. A.V. Turnbull and C. Rivier, *Proc Soc Exp Biol Med*, **215**, 1 (1997).
2. R. Chen, K.A. Lewis, M.H. Perrin and W.W. Vale, *Proc. Natl. Acad. Sci. (USA)*, **90**, 8967 (1993).
3. N. Vita, P. Laurent, S. Lefort, P. Chalon, J.M. Lelias, M. Kaghad, F.G. Le, D. Caput and P. Ferrara, *Febs Letters*, **335**, 1 (1993).
4. C.P. Chang, R.I. Pearce, S. O'Connell and M.G. Rosenfeld, *Neuron*, **11**, 1187 (1993).
5. M.H. Perrin, C.J. Donaldson, R. Chen, K.A. Lewis and W.W. Vale, *Endocrinology*, **133**, 3058 (1993).
6. D.E. Grigoriadis and E.B. De Souza, *Endocrinology*, **125**, 1877 (1989).
7. T.W. Lovenberg, C.W. Liaw, D.E. Grigoriadis, W. Clevenger, D.T. Chalmers, E.B. De Souza and T. Oltersdorf, *Proc. Natl. Acad. Sci. (USA)*, **92**, 836 (1995).
8. T. Kishimoto, R.V. Pearce II, C.R. Lin and M.G. Rosenfeld, *Proc. Natl. Acad. Sci. (USA)*, **92**, 1108 (1995).
9. M. Perrin, C. Donaldson, R. Chen, A. Blount, T. Berggren, L. Bilezikjian, P. Sawchenko and W. Vale, *Proc. Natl. Acad. Sci. (USA)*, **92**, 2969 (1995).
10. W.A. Kostich, A. Chen, K. Sperle and B.L. Largent, *Mol. Endocrinol.*, **12**, 1077 (1998).
11. F. Petraglia, P. Fl. M. Stomati, D.A. (
12. C.G. Kemp, R.J. (
13. D.T. Chalmers, T. Pharmacol. Sci.,
14. M.P. Stenzel-Poo Y Acad Sci, **780**,
15. L. Muglia, L. Jaco
16. G.W. Smith, J.-M Marchuk, C. Hau Neuron, **20**, 1093
17. T.M. Ramesh, I.J Abstr., **24**, 505 (1
18. C.B. Nemeroff, B
19. S.C. Heinrichs, T
20. H.C. Wu, K.Y. Ch
21. T. Skutella, J.C. (1998).
22. G. Liebsch, R. L Holsboer and A. M
23. S.C. Heinrichs, J. Pept, **71**, 15 (199
24. A.J. Dunn and C.)
25. G.P. Chrousos, A
26. P.J. Gilligan, P.R (1997).
27. T.Z. Baram and C
28. Y.L. Chen, R.S. Dunaikis, W.S. F 1749 (1997).
29. C. Chen, T.R. We
30. A.G. Arvanitis and
31. Y.L. Chen, WO 91
32. P.J. Gilligan, C. E American Chemic
33. T. Capris, D.J. MacKenzie, T.A. Chemistry Sympo
34. D.J. Wustrow, T MacKenzie, T.A. 8, 2067 (1998).
35. K. Wilcoxon, C. C Souza and J.R. I Chemistry, Anahei
36. L. He, P.J. Gillig; Shelton, M. Smith of Medicinal Cher
37. J.P. Beck, A.G. / Fitzgerald, R. Z Meeting, Las Vega
38. C. Chen, R. Dagr Moran, T.R. Web (1996).
39. P.E. Aldrich, A.G D.E. Grigoriadis, Wasserman, WO
40. Y.L. Chen, WO 91
41. J.R. McCarthy, J Huang, Z. Liu, Y.f
42. R. Bakhavatchal; WO 97/35539 (19

ous system and
lal stressors and
sauvagine and
gous to those of
the two agonists
hus, the precise
eptides will likely
ubtype and CRF

CRF-BP

Yes
Yes
Yes
Yes

Yes
No
No
No

has substantially
a pharmaceutical
ort in this series
these include: 1)
iological roles of
the discovery of
mical distribution
ree fronts since
beit with mixed
h selective non-
r future.

i. (USA), 90, 8967

e, D. Caput and P.

1187 (1993).

ndocrinology, 133,

ars, E.B. De Souza

Acad. Sci. (USA),

P. Sawchenko and

1077 (1998).

11. F. Petraglia, P. Florio, R. Gallo, C. Salvestroni, M. Lombardo, A.D. Genazzani, C. Di Carlo, M. Stomati, D.A. G and P.G. Artini, *Horm Res*, **45**, 187 (1996).
12. C.G. Kemp, R.J. Woods and P.J. Lowry, *Peptides*, **19**, 1119 (1998).
13. D.T. Chalmers, T.W. Lovenberg, D.E. Grigoriadis, D.P. Behan and E.B. De Souza, *Trends Pharmacol. Sci.*, **17**, 166 (1996).
14. M.P. Stenzel-Poore, J.E. Duncan, M.B. Rittenberg, A.C. Bakke and S.C. Heinrichs, *Ann N Y Acad Sci*, **780**, 36 (1996).
15. L. Muglia, L. Jacobson and J.A. Majzoub, *Ann N Y Acad Sci*, **780**, 49 (1996).
16. G.W. Smith, J.-M. Aubry, F. Dellu, A. Contarino, L.M. Bilezikjian, L.H. Gold, R. Chen, Y. Marchuk, C. Hauser, C.A. Bentley, P.E. Sawchenko, G.F. Koob, W. Vale and K.-F. Lee, *Neuron*, **20**, 1093 (1998).
17. T.M. Ramesh, I.J. Karolyi, M. Nakajima, S.A. Camper and A.F. Seasholtz, *Soc. Neurosci. Abstr.*, **24**, 505 (1998).
18. C.B. Nemeroff, *Biol. Psychiatry*, **44**, 517 (1998).
19. S.C. Heinrichs, *Trends Pharmacol. Sci.*, (in press), (1999).
20. H.C. Wu, K.Y. Chen, W.Y. Lee and E.H.Y. Lee, *Neuroscience*, **78**, 147 (1997).
21. T. Skutella, J.C. Probst, U. Renner, F. Holsboer and C. Behl, *Neuroscience*, **85**, 795 (1998).
22. G. Liebsch, R. Landgraf, R. Gerstberger, J.C. Probst, C.T. Wotjak, M. Engelmann, F. Holsboer and A. Montkowski, *Regul. Peptides*, **59**, 229 (1995).
23. S.C. Heinrichs, J. Lapsansky, T.W. Lovenberg, E.B. De Souza and D.T. Chalmers, *Regul. Pept*, **71**, 15 (1997).
24. A.J. Dunn and C.W. Berridge, *Br. Res. Rev.*, **15**, 71 (1990).
25. G.P. Chrousos, *Ann. New York Acad. Sci.*, **851**, 388 (1998).
26. P.J. Gilligan, P.R. Hartig, D.W. Robertson and R. Zaczek, *Ann. Rep. Med. Chem.*, **32**, 41 (1997).
27. T.Z. Baram and C.G. Hatalski, *Trends Neurol. Sci.*, **21**, 471 (1998).
28. Y.L. Chen, R.S. Mansbach, S.M. Winter, E. Brooks, J. Collins, M.L. Corman, A.R. Dunaiskis, W.S. Faraci, R.J. Gallaschun, A. Schmidt and D.W. Schulz, *J. Med. Chem.*, **40**, 1749 (1997).
29. C. Chen, T.R. Webb, J.R. McCarthy and K.M. Wilcoxon, *WO 97/29109* (1997).
30. A.G. Arvanitis and R.J. Chorvat, *WO 98/03510* (1998).
31. Y.L. Chen, *WO 98/08847* (1998).
32. P.J. Gilligan, C. Baldauf, A. Cocuzza, D. Chidester, L. Fitzgerald, R. Zaczek and H. Shen, *American Chemical Society National Meeting*, Boston, MA, 1998, *MEDI 135*.
33. T. Capiaris, D.J. Wustrow, M.R. Rubin, J.A. Knobelsdorf, H. Akunne, M.D. Davis, R. MacKenzie, T.A. Pugsley, K.T. Zoski, T.G. Heffner and L.D. Wise, *26th National Medicinal Chemistry Symposium*, Omni Richmond Hotel, Richmond VA, 1998, *D*.
34. D.J. Wustrow, T. Capiaris, R. Rubin, J.A. Knobelsdorf, H. Akunne, M.D. Davis, R. MacKenzie, T.A. Pugsley, K.T. Zoski, T.G. Heffner and L.D. Wise, *Bioorg Med Chem Lett*, **8**, 2067 (1998).
35. K. Wilcoxon, C. Chen, C. Huang, M. Haddach, Y.-F. Xie, L. Wing, D.E. Grigoriadis, E.B. De Souza and J.R. McCarthy, *American Chemical Society Abstracts, Division of Medicinal Chemistry*, Anaheim, CA, 1999, *MEDI 02*.
36. L. He, P.J. Gilligan, R. Zaczek, L. Fitzgerald, N. Kalin, J. McElroy, J. Saye, H. Shen, S. Shelton, M. Smith, G. Trainor and P. Hartig, *American Chemical Society Abstracts, Division of Medicinal Chemistry*, Anaheim, CA, 1999, *MEDI 04*.
37. J.P. Beck, A.G. Arvanitis, A.J. Cocuzza, D.R. Chidester, M.A. Curry, J.T. Rescinito, L.W. Fitzgerald, R. Zaczek and J.C. Calabrese, *American Chemical Society National Meeting*, Las Vegas, NV, 1997, *MEDI 094*.
38. C. Chen, R. Dagnino Jr., E.B. De Souza, D.E. Grigoriadis, C.Q. Huang, K.I. Kim, Z. Lui, T. Moran, T.R. Webb, J.P. Whitten, Y.F. Xie and J.R. McCarthy, *J. Med. Chem.*, **39**, 4358 (1996).
39. P.E. Aldrich, A.G. Arvanitis, R.S. Cheeseman, R.J. Chorvat, T.E. Christos, P.J. Gilligan, D.E. Grigoriadis, C.N. Hodge, P.J. Krenitsky, E.L. Scholfield, S.W. Tam and Z.R. Wasserman, *WO 95/10506* (1995).
40. Y.L. Chen, *WO 95/33750* (1995).
41. J.R. McCarthy, J.P. Whitten, T.R. Webb, J.Y. Ramphal, D.E. Grigoriadis, C. Chen, C.Q. Huang, Z. Liu, Y.F. Xie and R. Dagnino, *WO 96/39400* (1996).
42. R. Bakthavatchalam, A.G. Arvanitis, J.P. Beck, G.A. Cain, R.J. Chorvat and P.J. Gilligan, *WO 97/35539* (1997).

43. R. Bakhavatchalam, A.G. Arvanitis, P.J. Gilligan, R.E. Olson, D.W. Robertson, G.L. Trainor, S.C. Smith, L.W. Fitzgerald, R. Zaczek, H. Shen and D.D. Christ. 216 ACS National Meeting, Boston, MA, 1998, MEDI 134
44. J.R. McCarthy, S.C. Heinrichs and D.E. Grigoriadis, *Current Pharmaceutical Design*, **5**, 247 (1999).
45. M. Mclean, A. Bisits, J. Davies, R. Woods, P. Lowry and R. Smith, *Nature Medicine*, **1**, 460 (1995).
46. R. Smith, *Scientific American*, *March*, 68 (1999).
47. J.P. Beck, P. Tivimahaissoon, B.K. Folmer, M.A. Curry, L.W. Fitzgerald, P.J. Gilligan, R. Zaczek, D.W. Robertson and W. Marshal. American Chemical Society Abstracts, Division of Medicinal Chemistry, 1999, MEDI 01
48. C.Q. Huang, M. Haddach, C. Chen, K.W. Wilcoxon, Y.-F. Xie, D. Grigoriadis, E.B. De Souza and J.R. McCarthy. American Chemical Society, Division of Medicinal Chemistry, Anaheim, CA, 1999, MEDI 003
49. C.N. Hodge, P.E. Aldrich, Z.R. Wasserman, C.H. Fernandez, G.A. Nemeth, A. Arvanitis, R.S. Cheeseman, R.J. Chorvat, E. Ciganek, T.E. Christos, P.J. Gilligan, P. Krenitsky, E. Scholfield and P. Strucely, *J Med Chem*, **42**, 819 (1999).
50. A.G. Arvanitis, P.J. Gilligan, R.J. Chorvat, R.S. Cheeseman, T.E. Christos, R. Bakhavatchalam, J.P. Beck, A.J. Cocuzza, F.W. Hobbs, R.G. Wilde, C. Arnold, D. Chidester, M. Curry, L. He, A. Hollis, J. Klaczekiewicz, P.J. Krenitsky, J.P. Rescinito, E. Scholfield, S. Culp, E.B. De Souza, L. Fitzgerald, D. Grigoriadis, S.W. Tam, Y.N. Wong, S.M. Huang and H.L. Shen, *J Med Chem*, **42**, 805 (1999).
51. R.J. Chorvat, R. Bakhavatchalam, J.P. Beck, P.J. Gilligan, R.G. Wilde, A.J. Cocuzza, F.W. Hobbs, R.S. Cheeseman, M. Curry, J.P. Rescinito, P. Krenitsky, D. Chidester, J.A. Yarem, J.D. Klaczekiewicz, C.N. Hodge, P.E. Aldrich, Z.R. Wasserman, C.H. Fernandez, R. Zaczek, L.W. Fitzgerald, S.M. Huang, H.L. Shen, Y.N. Wong, B.M. Chien, C.Y. Quon and A. Arvanitis, *J Med Chem*, **42**, 833 (1999).
52. T.E. Christos and A. Arvanitis, *Expert Opinion in Therapeutic Patents*, **8**, 143 (1998).
53. D.R. Luthin, A.K. Rabinovich, D.R. Bhuralkar, K.L. Youngblood, R.A. Bychowski, D.S. Dhanoa and J.M. May, *Bioorganic & Medicinal Chemistry Letters*, **9**, 765 (1999).
54. J.P. Whitten, J.R. McCarthy, Z. Liu, C.Q. Huang, P.E. Erickson, D.P. Behan, Y.F. Xie and R.F. Lowe, WO 97/45421 (1997)
55. C.H. Mitch and S.J. Quimby, WO 98/51312 (1998)
56. J. Rivier, C. Rivier, R. Galyean, A. Miranda, C. Miller, A.G. Craig, G. Yamamoto, M. Brown and W. Vale, *J. Med. Chem.*, **36**, 2851 (1993).
57. J. Rivier, S.L. Lahrachi, J. Gulyas, J. Erchegeyi, S.C. Koerber, A.G. Craig, A. Corrigan, C. Rivier and W. Vale, *J Med Chem*, **41**, 2614 (1998).
58. S.W. Sutton, D.P. Behan, L.L. Sabine, R. Kaiser, A. Corrigan, P. Lowry, E. Potter, M.H. Perrin, J. Rivier and W. Vale, *Endocrinology*, **136**, 1097 (1995).
59. E.T. Wei, H.A. Thomas, H.C. Christian, J.C. Buckingham and T. Kishimoto, *Peptides*, **19**, 1183 (1998).
60. F. Menzaghi, R.L. Howard, S.C. Heinrichs, W. Vale, J. Rivier and G.F. Koob, *J. Pharmacol. Exp. Ther.*, **269**, 564 (1994).
61. S.C. Heinrichs, E.A. Vale, J. Lapsansky, D.P. Behan, L.V. McClure, N. Ling, E.B. De Souza and G. Schulteis, *Peptides*, **18**, 711 (1997).
62. A. Ardati, J. Gottowik, S. Henriot, R.G. Clerc and G.J. Kilpatrick, *J. Neurosci. Meth.*, **80**, 99 (1998).
63. M. Spina, E. Merlo-Pich, R.K. Chan, A.M. Basso, J. Rivier, W. Vale and G.F. Koob, *Science*, **273**, 1561 (1996).
64. D.N. Jones, R. Kortikaas, P.D. Slade, D.N. Middlemiss and J.J. Hagan, *Psychopharmacol.*, **138**, 124 (1998).

Introduction - is the deposit the walls of the cleavage to self-aggregate pathogenesis three familial mutations, all for AD, which fibrillized A β overexpressing approach for a peptide. For that blocking Furthermore, form A β , have binding to the in AD pathologic sulfate prote aggregation is the symptoms techniques for amyloid inhibit update on the

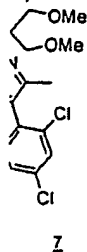
BI

Rationale - It is useful to consider how it occurs along a relatively stable structure and intermediate the activity is intermediate protein folding a different inhibitory. Therefore in the pathologic fibrillization it

of mental illness. ion of individuals ed cortisol levels l, thus supporting due to abnormal ent of depressed led in a decrease tment as well as This observation ays may underlie , which case CRF tic benefit. In ould conceivably ircuits (25).

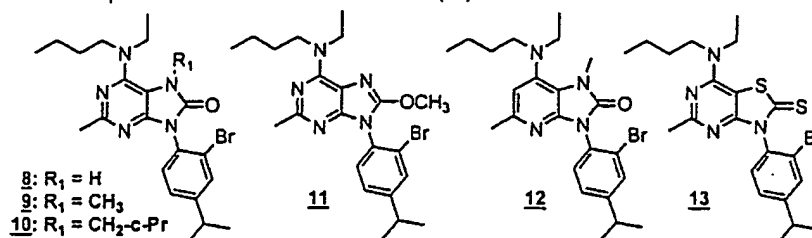
CRF₁ RECEPTOR ANTAGONISTS

block the CRF₁ and ACTH release en administered binding to CRF₁ ood-brain-barrier. iced elevations in : blocked in the antagonists have) suggesting that ystemically, also ates the ability of This compound) and in the same mparison of the obutyl analog (2) $IC_{50} = 5.5$ nM) was

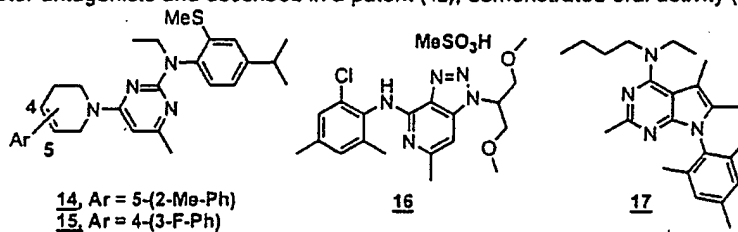


of pyrazolo[1,5-a]s-triazine of the first patent ed (30, 31). The 45 hours and oral

bioavailability of 33.1 % (32). A SAR study of similar compounds found the most potent analog, **5**, to contain a 2,4-dichlorophenyl ring and a N-propyl N-cyclopropylmethyl amino side chain at the 7-position ($K_i = 3.2$ nM) (33, 34). From a SAR study, NBI 30545 (**6**) was selected for further study ($K_i = 2.8$ nM) and demonstrated efficacy in the CRF-induced locomotor activity model in mice (35). DMP-696, (**7**), ($K_i = 1.7$ nM) an orally active CRF receptor antagonist from the related pyrazolo[1,5-a]s-triazine series (30), demonstrated 50% oral bioavailability in dogs with $t_{1/2} = 33.4$ hours and $t_{1/2} = 15$ hours in rhesus monkeys (p.o.). No endocrine, cardiovascular, GI, or pulmonary effects were noted at doses 30-fold higher than those effective in the rat situational anxiety assay. Furthermore, the potency of the molecule improves on chronic administration (36).

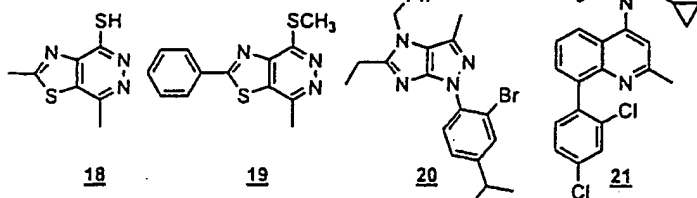


Certain N-7-alkyl-N-9-aryl-8-oxopurines, as well as structurally related imidazopyridine-2-ones, are CRF₁ receptor antagonists (37). The importance of a small N-7 alkyl group for tight binding of 8-oxopurines to the CRF₁ receptor is seen by comparing **8** ($K_i = 890$ nM), **9** ($K_i = 4.9$ nM) and **10** ($K_i = 117$ nM). O-Methylation of the 8-oxo group on **8**, to yield **11**, provides a more potent antagonist, ($K_i = 1.5$ nM) while the 1-deaza analog **12** ($K_i = 4.0$ nM) was equipotent to **9**. Thiazolo[4,5-d]pyrimidine thione **13** ($K_i = 4.1$ nM) contains the favored 2-bromo-4-isopropyl substituted phenyl ring common to a number of tight binding CRF receptor antagonists. CRA0165 (**14**) ($IC_{50} = 12.7$ nM) and CRA1000 (**15**) ($IC_{50} = 10.5$ nM) were found to have good affinity for the CRF₁ receptor. Oral administration of these compounds to mice and rats with stress-induced and CRF-induced anxiogenic-like activity ameliorated these effects in the 0.1 to 10 mg/kg range. Several anilino-aminopyrimidine-based CRF receptor antagonists have previously been reported (38-41). DMP 695 (**16**), obtained from the N-aryl aminotriazolopyrimidine series of CRF receptor antagonists and described in a patent (42), demonstrated oral activity (43).



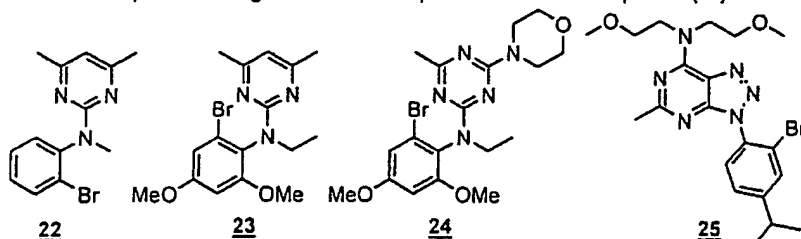
While a wide variety of anti-stress, anxiolytic and anti-depressant actions of small molecule CRF₁ receptor antagonists have been recently reported (44), one example which has received much attention concerns the fact that regulation of CRF levels may correlate with the length of pregnancy (45). In particular, plasma CRF levels increase exponentially as gestation advances beyond about 16 weeks and could possibly serve as a good predictor of delivery. Noting that women with the highest CRF levels were most likely to deliver prematurely, these investigators have posulated that CRF receptor blockade would act to delay parturition. In support of this hypothesis, infusion of antalarmin (**17**) to pregnant sheep delays delivery of lambs

until infusion is discontinued (46). Thiazolopyridazines **18** and **19** were reported by the same group to be promising leads in the development of CRF receptor agonists.



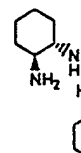
The imidazo[4,5-c]pyrazole **20** ($K_i = 4$ nM) is the most potent CRF receptor antagonist in a SAR study around this 5-5 bicyclic series (47). A SAR study on the contrasting 6-6 bicyclic 8-arylquinolines, represented by **21**, ($K_i = 0.5$ nM) reported a number of analogs with K_i values under 1 nM (48).

The screening lead **22** ($K_i = 5700$ nM) was optimized by systematic SAR studies that assisted in the definition of a pharmacophore (49) that is similar to a model previously proposed (38). Conformational preferences and barriers to rotation of anilino-aminopyrimidine **23** were determined by semiempirical methods, X-ray and variable temperature NMR spectroscopy. The study determined the active conformation of the anilino-pyrimidines and led to the synthesis of additional anilino-amino-based pyrimidine CRF receptor antagonists as well as SA627 (**24**) ($K_i = 32$ nM) (50). Compound **24** was evaluated in dog at 5 mg/kg (iv, po). The oral bioavailability was 20 % and the mean peak oral plasma level was 730 nM at 0.5 hours. However, this compound was not advanced further since members of the bicyclic triazolo[4,5-d]pyrimidine series were more promising. From an extensive SAR study, SC241 (**25**) ($K_i = 3.7$ nM) was believed to have properties necessary for a potential therapeutic agent. Further pharmacological studies are planned for this compound (51).



In the past two years, since the last review on CRF in this series (26), the number of patents and applications combined has nearly doubled. However, there is a scarcity of receptor binding data published in these patents. Two reviews have appeared that include these patents (44; 52). The more recent review summarizes generic structures and gives examples of structures claimed for all the small molecule CRF receptor antagonist patents (44).

Oxo-7H-benzo[e]spermidine-4-carboxamide **26** displaced radiolabeled o-CRF from both CRF₁ ($K_i = 110$ nM) and CRF₂ ($K_i = 20$ nM) receptors (53). Compound **26** is the most potent analog in a series of the first reported mixed CRF₁/CRF₂ receptor antagonists. The compound antagonized CRF-stimulated cAMP formation and CRF-stimulated corticotropin release from rat pituitary *in vivo*, suggesting an antagonist. The binding selectivity profile was determined for **26** at a number of different receptors. The compound showed weak binding to NPY Y1 ($K_i = 4200$ nM), D₂ ($K_i = 3200$ nM) and 5-HT₇ ($K_i = 1600$ nM), but binding $> 10,000$ nM against the other receptors screened.



The first pal structure **27**, as treated with 1 m slight improved acquisition traini structures, were appeared in the

CRF RECEI

Construction of affords a variety discrete and sep CRF receptor bi instance, the cyc lactam ring (e.g. the equivalent i constraint that evidence was p peptide agonists the tertiary struc by the aminote carboxyterminus physiochemical i 41) and rat or h helical CRF(9-4' and peptides su (Table 1). This : (59) which can gi

In Vivo Efficacy binding profile f functional action competitive CRF both block CRF₁ fact that α -helical actions (60) which protein. This su indirect CRF ag rat/human CRF receptor agonist urocortin (CRF t dissociation from binding protein > the dissociation irreversible bindi *in vivo* efficacy c also been exam motor activity, e



PERGAMON

Journal of Psychiatric Research 33 (1999) 181–214

JOURNAL OF
PSYCHIATRIC
RESEARCH

The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety

F. Holsboer*

Max Planck Institute of Psychiatry, Kraepelinstr. 10, D-80804 Munich, Germany

Accepted 26 October 1998

Abstract

Neuroendocrine studies strongly suggest that dysregulation of the hypothalamic–pituitary–adrenocortical (HPA) system plays a causal role in the development and course of depression. Whereas the initial mechanism resulting in HPA hyperdrive remains to be elucidated, evidence has emerged that corticosteroid receptor function is impaired in many patients with depression and in many healthy individuals at increased genetic risk for an depressive disorder. Assuming such impaired receptor function, then central secretion of CRH would be enhanced in many brain areas, which would account for a variety of depressive symptoms. As shown in rats and also in transgenic mice with impaired glucocorticoid receptor function, antidepressants enhance the signaling through corticosteroid receptors. This mechanism of action can be amplified through blocking central mechanisms that drive the HPA system. Animal experiments using antisense oligodeoxynucleotides directed against the mRNA of both CRH receptor subtypes identified the CRH₁ receptor as the mediator of the anxiogenic effects of CRH. Studies in mouse mutants in which this receptor subtype had been deleted extended these findings as the animals were less anxious than wild-type mice when experimentally stressed. Thus, patients with clinical conditions that are causally related to HPA hyperactivity may profit from treatment with a CRH₁ receptor antagonist. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

For more than 100 years psychopharmacology has been shaped by compounds that have emerged from organic chemistry laboratories and whose systemic effects have then been studied by careful but unsystematic analysis. For example, in 1832 the German chemist Justus von Liebig synthesized chloral hydrate from ethanol and chlorinated lime. A few years later, in Boston, the American physician Charles T. Jackson accidentally observed the sedating effect of ether. This led researchers to test sedating and narcotic effects of other gaseous compounds such as chloroform. The German pharmacologist Oscar Liebreich postulated that chloroform might derive from chloral hydrate by degradation in blood, suggesting that chloral hydrate might be a sedative drug that could be administered orally. The first clinical trials, conducted in the department of psychiatry of the Charité Hospital in Berlin, confirmed the postulated sedative effect, although it turned out that it was not the decomposition of chloral hydrate into chloroform that caused sedation. Similar cases in which potential clinical applications for newly

developed chemical compounds were investigated led to the description of clinical indications for barbiturates and phenothiazines. A prominent serendipitous finding, made by the psychiatrist Roland Kuhn in Switzerland in the mid-50s, was for instance the discovery that heterocyclic compounds such as iminodibenzyl derivatives, to which imipramine belongs, have the property to act as antidepressants. All these developments have several characteristics in common: (1) The compounds were synthesized without any anticipation of their clinical utility; (2) their mode of action was unknown and the mechanism thought to be involved in the obvious clinical efficacy often turned out to be wrong; and (3) the clinical efficacy stimulated hypotheses about the causality of the respective disorder. For example, the Australian psychiatrist John Cade believed that lithium salts act through diathesis of uric acid, which produces psychosis. This hypothesis did not stand the test of time. Another pathogenetic hypothesis, departing from the pharmacology of antidepressants, postulated a central deficiency of bioavailable norepinephrine and serotonin. Thus, it was the antidepressants' mechanism of action itself that prompted the formulation of the biogenic amine deficiency hypothesis as put forward by Joe Schildkraut in Boston and Alec Coppen in London, and it may be said that no other hypothesis has influenced the development of anti-

* Tel.: +49 89 30622 220; fax: +49 89 30622 483; e-mail: holsboer@mpipsy.klin.mpg.de.

depressants to a similar extent. In this article, depression will serve as an example to illustrate that the classic 'from bench to bed' approach is now becoming more complex, as clinical and preclinical research identify central pathological mechanisms that can provide specific drug targets. One example of such a 'from bed to bench and back' strategy is the close interrelation between the dysregulation of the hypothalamic–pituitary–adrenocortical (HPA) activity in individuals with depression, the progression into depression, the action of current antidepressants, and the development of new drugs targeting HPA regulation.

2. Clinical evidence for CRH hyperactivity in depression

In response to acute physical or psychological stress, parvocellular neurons of the paraventricular hypothalamus (PVN) produce increased amounts of corticotropin-releasing hormone (CRH), which is released into portal vessels activating secretion of corticotropin (ACTH) from anterior pituitary cells. In turn, ACTH enters the circulation and elicits glucocorticoids from the adrenal gland. This rapid HPA activation can be life-sustaining because of the metabolic effect of elevating blood glucose levels. However, other stress-related responses needed for life-sustaining adaptations encompass a number of behavioral reflexes elicited by activation of the HPA system, presumably by an increase in CRH release (Fig. 1).

The many checks and balances of the HPA system serve to counter-regulate the stress-elicited activation. In individuals with major depression, however, there is sustained hyperactivity of the HPA system. In the following the evidence is reviewed that points to an unrestrained CRH hyperdrive in these patients, which would explain many of their symptoms.

Shortly after the group led by W. Vale at the Salk Institute in La Jolla, California, had isolated, sequenced and characterized CRH, synthetic ovine, rat and human neuropeptide probes became available for human studies (Vale et al., 1981). After a bolus of ovine CRH, patients with depression had ACTH responses that were indistinguishable from those of normal controls when the CRH was injected in the morning (Holsboer, 1983; Holsboer et al., 1984a) and attenuated when it was injected in the evening, when the HPA system is quiescent (Gold et al., 1984). Whereas the ACTH response to ovine CRH produces prolonged and higher peak concentrations than human CRH (hCRH), the latter more closely simulates the physiological condition (Orth, 1992). When hCRH is injected in the evening, it also produces attenuated ACTH responses in depressed patients (Holsboer et al., 1984b, 1986). These first studies and their numerous replications suggested that elevated

corticosteroid levels in combination with desensitized CRH receptors at corticotrophic cells might restrain the releasable amount of ACTH. Support for this view was provided by studies using metyrapone, which inhibits cortisol synthesis by blocking hydroxylation at the C11 position, showing that the ACTH blunting is avoided when plasma cortisol concentrations are lowered (von Bardeleben et al., 1988; Lisansky et al., 1989). A more recent study by Young et al. (1995) suggested that the evidence for CRH receptor desensitization at corticotrophic cells is mute as they found a 3-fold higher β -endorphin response to CRH in depressed patients pretreated with metyrapone than in those without pretreatment. Both β -endorphin and ACTH are synthesized and released from the pituitary after corticotrophic CRH receptor activation. In retrospect, the conclusions drawn from these studies are perhaps limited, as the enzyme block induced by metyrapone generates many other corticosteroid derivatives with neural activities of their own (Paul and Purdy, 1992; Rupprecht, 1997). For example, 11-deoxycorticosteroids are excessively increased after metyrapone, and studies by Patchev et al. (1994a, 1997) suggested that tetrahydrodeoxycorticosterone (THDOC), a major metabolite of 11-deoxycorticosterone, also interferes with CRH release and action.

Indirect evidence in favor of CRH receptor desensitization comes from studies with patients having panic attacks, in whom under non-panic conditions CRH-elicited ACTH is blunted despite normal plasma corticosterone levels at baseline. These studies were interpreted as indicative of CRH receptor desensitization, secondary to episodic increases of CRH during panic (Roy-Byrne et al., 1986; Holsboer et al., 1987a).

Although it seems difficult to determine whether blunted ACTH response is caused either by elevated corticosteroids or by desensitized CRH receptors, it is important to note that these are two closely related phenomena. To further explore these interdependencies, we pretreated both depressed patients and matched healthy controls with dexamethasone and observed that in the depressed patients the ACTH and cortisol responses were exaggerated whereas in the controls the ACTH and cortisol elevations were only mute (von Bardeleben and Holsboer, 1989, 1991). Age was found to be an important factor in enhancing ACTH and cortisol release (Heuser et al., 1996), and estradiol supplementation markedly attenuated the hormonal response to the dexamethasone-CRH test in postmenopausal women (Kudielka et al., unpublished observations). Systematic analysis of a broad data base revealed that this combined dexamethasone-(h)CRH test identified HPA dysregulation with high sensitivity in patients with affective disorders (Heuser et al., 1994). The explanation for the suppressing effect of basal cortisol on CRH-elicited ACTH versus the exaggerated ACTH response to low-dose dexamethasone pretreatment is based on the intriguing pharmacological

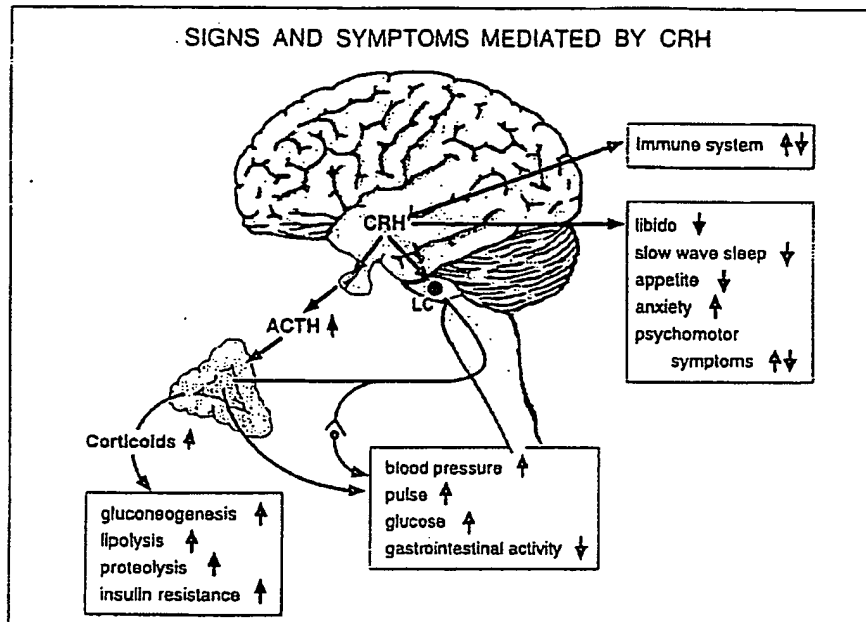


Fig. 1. Animal studies in which CRH was injected intracerebroventricularly or CRH synthesis was disrupted (knockout mice or antisense oligodeoxynucleotide treatment of rats) or CRH receptor function was manipulated through antagonists, gene therapy or gene targeting are in accord with indirect clinical evidence that many signs and symptoms present in depression can be attributed to enhanced secretory activity of central CRH.

differences between the two steroids: (1) Dexamethasone binds primarily to glucocorticoid receptors (GR), whereas cortisol also binds to GRs at corticotrophic and hypothalamic sites. In the hippocampus, however, which is also strongly involved in HPA regulation, cortisol, unlike dexamethasone, binds preferably to mineralocorticoid receptors (MRs) and only at high concentrations, for instance during stress, also to GRs; (2) in plasma, cortisol but not dexamethasone is bound to corticosteroid-binding globulin, which directs the action of dexamethasone to corticotrophic GRs; (3) the perhaps most important difference between cortisol and dexamethasone stems from the fact that dexamethasone is a substrate for the multidrug-resistant (mdr) 1a P-glycoprotein (Bourgeois et al., 1993), which is expressed in the apical membranes of endothelial cells of the blood-brain barrier (Cordon-Cardo et al., 1989). At low dosages, this mdr 1a P-glycoprotein acts as a pump, limiting passage of xenobiotic agents including dexamethasone from peripheral circulation into the brain (Schinkel et al., 1995). Meijer et al. (1998) have recently demonstrated that dexamethasone retention in the hippocampus and hypothalamus was much higher in mutant mice with a disrupted mdr1a gene, reaching levels in the order of pituitary retention, than in wild-type mice.

The consequence of the different mode of action of cortisol and dexamethasone is that in depressed patients

pretreated with a low dose of dexamethasone which, as already pointed out, acts primarily at the pituitary to suppress ACTH and, in turn, cortisol secretion, a deprivation of central natural ligands for GR and MR occurs. Because this loss is due to the limited access of dexamethasone to the brain, central regulatory sites sense this situation as adrenocortical insufficiency or a transient chemical adrenalectomy. In response, central HPA stimulants (ACTH secretagogues), mainly CRH and vasopressin (AVP), are hypersecreted and released via the median eminence into portal circulation. When CRH is injected under this condition, it synergizes with endogenously released AVP to override dexamethasone-induced suppression at corticotrophic cells. This interpretation was based on the finding that neither CRH nor AVP, when given alone, is capable of producing a major ACTH escape from dexamethasone suppression in controls (von Bardeleben et al., 1985; Wiedemann and Holsboer, 1997). However, when both neuropeptides were administered to dexamethasone-pretreated healthy controls, the resulting ACTH and cortisol levels were comparable to those of depressed patients, suggesting that among depressives not only CRH but also AVP is hypersecreted (von Bardeleben et al., 1985; von Bardeleben and Holsboer, 1989). Further evidence in support of this hypothesis was recently provided by the group of Dick Swaab in Amsterdam, who found increased num-

bers of both CRH-secreting neurons and CRH neurons that coexpressed AVP mRNA in the hypothalami of depressed patients (Raadsheer et al., 1994; Purba et al., 1996) (Fig. 2). In line with this is the report by Nemeroff et al. (1988) of a decrease in the number of CRH binding sites in the frontal cortex of depressed patients who committed suicide. This has been interpreted as an adaptive (homologous) down-regulation in response to increased CRH secretion. Two groups in England were unable to reproduce these findings, however, and could not confirm changes in CRH immunoreactivity or CRH binding sites in the cortices of depressed suicides (Charlton et al., 1988; Hucks et al., 1997; Leake et al., 1990), which probably reflects methodological difficulties that are not unlikely to be encountered in such studies. For example, the impact of the different violent methods used for committing suicide or the type of antidepressant treatment may be among the more important confounds.

Another line of evidence that CRH is not only a determinant of neuroendocrine signs of depression but is also causally involved in depressive psychopathology emerged from the work of Charles Nemeroff and colleagues in Atlanta, U.S.A. This group conducted a series of studies in which the CRH concentration in the cerebrospinal fluid (CSF) was measured by radioimmunoassay in drug-free depressed patients, patients in other diagnostic categories, and controls (Nemeroff et al., 1984; Banki et al., 1987; Arato et al., 1989). These and numerous other studies confirmed that CRH is elevated in the CSF of

patients with severe depressive illness, and importantly, that after successful drug treatment CSF levels of CRH had decreased again (De Bellis et al., 1993). Several other studies could not substantiate these CRH elevations in the CSF of depressives, however (Molchan et al., 1993; Pitts et al., 1995; Geraciotti et al., 1997). In this context, several factors need to be considered. The group led by Nemeroff used patients who were more acutely ill, and their studies and those of others indicate that the likelihood of CRH elevations increases with the presence of peripheral signs of HPA overactivity. In contrast, patients with eucortisolemic depression as studied by Geraciotti et al. (1992, 1997) do not seem to have elevated CRH concentrations in the CSF, which calls for studies investigating the extent to which peripheral cortisol concentrations may influence the activity of CRH neurons through actions on central CRH neurons. In rats, Swanson and Simmons (1989) showed that corticosteroids may activate CRH expression in the dorsal-parvocellular part of the hypothalamic PVN. From there, hypothalamic spinal projections of CRH neurons emerge, which suggests that CRH in the CSF is possibly a reflection of HPA activity, with cortisol acting as a stimulus for spinal CRH.

Taking all these clinical studies together, it seems fair to say that the CRH hypothesis is well founded, although several questions remain open. Of course, the limits of clinical examinations call for preclinical studies in animal models and basic studies at the cellular and molecular level to better understand how CRH is regulated and

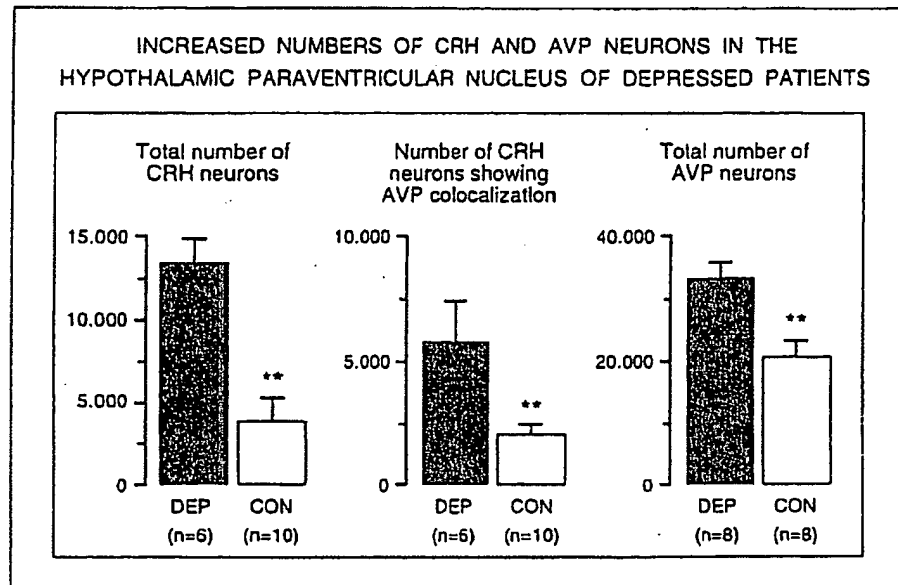


Fig. 2. Patients with major depression have an increased number of CRH and arginine vasopressin (AVP) neurons and also increased co-packaging of CRH and AVP in PVN neurons, which explains the excessive HPA activity in these patients as both neuropeptides synergize their actions at pituitary CRH receptors. DEP = depressed patients; CON = controls (adapted from Raadsheer et al., 1994 and Purba et al., 1996).

which regulatory elements might serve as potential drug targets of the HPA system.

3. Behavioral effects of CRH in animals

Only a few other neuropeptides have been studied more extensively than CRH with regard to their behavioral effects. Several reviews covering this issue have been published (Koob and Bloom, 1985; Dunn and Berridge, 1990; Holsboer et al., 1992; Owens and Nemeroff, 1992), which is why only a brief summary will be given of the main evidence that CRH acts as a mediator of affective symptoms.

Neuroanatomical studies strongly suggest that CRH not only accounts for neuroendocrine adaptations to stress, but that it may also have numerous other effects as CRH neurons have a widespread but selective distribution throughout the central nervous system (CNS). As shown by Swanson and Simmons (1989) and Merchenthaler (1994), the hypothalamic PVN is the major site of CRH-containing cell bodies. These cell bodies send axon terminals to the capillaries of the median eminence, from where CRH enters portal circulation to regulate proopiomelanocortin (POMC)-derived peptide (mainly ACTH and β -endorphin) synthesis and secretion from pituitary corticotrophs. Other CRH neurons of PVN origin project to the brainstem and spinal cord, both of which contain CRH cell bodies and influence behavioral activity and autonomic function. Spinal cord CRH neurons may also modulate sensory input through ascending pathways and, additionally, may represent preganglionic neurons that modulate sympathetic outflow.

The central nucleus of the amygdala (CeA) and the bed nucleus of the stria terminalis (BNST) contain large and discrete populations of CRH perikarya. Whereas CRH neurons from the BNST project to the brainstem, CRH neurons in the CeA send terminals to parvocellular regions of the PVN. These morphological findings together with the findings that CRH fibers interconnect the CeA, PVN and BNST clearly indicate that autonomic and neuroendocrine actions of CRH are functionally intertwined. A CRH involvement in complex behaviors is suggested by the presence of CRH interneurons, localized to layers II and III of the cortex. Although CRH-containing neurons are present throughout the neocortex, particularly high densities are found in the prefrontal cortex, emphasizing a role of CRH in cognitive processes.

Most studies exploring behavioral effects of CRH in animals have used intracerebroventricular (icv) or site-specific injections of CRH, and all agree that CRH mediates numerous anxiogenic and fear-related aspects of stress. When injected into rats or mice in a novel environment, CRH increases grooming and freezing behavior and decreases the number of approaches to a food pellet (Britton et al., 1982; Sutton et al., 1982).

In the conflict test, CRH suppresses both punished and nonpunished responding, which is another indication of anxiety-related behavior (Britton et al., 1986). Other studies in rats demonstrated a CRH-induced potentiation of acoustic startle (Swerdlow et al., 1989), a suppression of social interaction (Dunn and File, 1987), and an increase in stress-induced freezing behavior (Sherman and Kalin, 1988). Furthermore, symptoms of behavioral despair were observed in investigations of adult rhesus monkeys (Kalin, 1990). Most of these behavioral effects were blocked by α -helical CRH_{41–49}, which acts as a CRH receptor antagonist, but has intrinsic effects by itself (see below). Administration of CRH to neurons of the locus coeruleus (LC) has excitatory effects (Valentino et al., 1983), which prompted Butler et al. (1990) to study the behavioral effects of CRH microinfusion into the LC. These authors used a modified version of the open field test to monitor anxiogenic effects and showed that with increasing CRH dosages the exploratory behavior decreased, while the time spent in the darkened compartment increased. This dose-dependent surge in anxiety-related behavior was accompanied by increased norepinephrine turnover in forebrain areas, to which noradrenergic neurons project.

All these experiments were built upon increases in anxiety-related behavior as elicited by high CRH dosages in normal rats. More recently, the possibility of studying the effects of decreased CRH neurotransmission by administering antisense oligodeoxynucleotides (ODNs) corresponding to the start-coding region of CRH mRNA was employed. With this technique, translation of CRH mRNA into CRH is suppressed, enabling assessment of whether the decreased CRH concentrations lead to a suppression of anxiety under basal and stress conditions. Skutella et al. (1994a,b) used the shuttle-box experiment and the social defeat paradigm to measure the anxiety-related behavior in rats by assessing the entries into and time spent on the open arms of the elevated plus-maze. As expected, anxiety-related behavior was decreased by CRH antisense ODN probes and the specificity of this treatment with regard to CRH targeting was confirmed by decreases in CRH concentrations in the PVN and reduced ACTH plasma levels. A different approach was used by Stenzel-Poore et al. (1994), who bred transgenic mice overexpressing CRH. These mice have deficits in emotionality and can serve as genetic models of anxiogenic behavior. In addition to increased anxiety-related behavior, which is reversible after administration of a CRH receptor antagonist, CRH transgenic mice also show memory impairments (Heinrichs et al., 1996). Moreover, CRH is also capable of inducing a number of other behavioral changes that can be extrapolated to human affective disorders. Among these are CRH-elicited changes in sleep as measured by electroencephalography. If CRH is administered centrally to rats or peripherally to healthy controls, slow wave sleep,

the part of sleep that is believed to have recuperating effects, is decreased and sleep onset-related growth hormone release is blunted (Ehlers et al., 1986; Holsboer et al., 1988). Both phenomena are also common in individuals with depression (Steiger and Holsboer, 1997).

Psychomotor changes are also frequent in these patients, and CRH can increase locomotor activity as demonstrated by studies in rats (Sutton et al., 1982). Decreased sexual drive is another cardinal symptom of depression, and therefore it is important that reproductive behavior is potently inhibited by central administration of CRH (Sirinathsinghji, 1986). Furthermore, transgenic mice overexpressing CRH exhibit reproductive deficits due to decreased hypothalamic-pituitary-gonadal activity, which can be interpreted as being responsible for decreased sexual interest.

Most patients with major depression have a loss of appetite. Consequently, weight loss is a frequent symptom in these patients, and chronically stressed people often become anorectic. Chronic icv injections of CRH into rats produces weight loss because of decreased food intake, suggesting that a CRH excess is also involved in stress-associated anorexia. However, these and many other studies have used high dosages of CRH, and there is no doubt that anorexia, sleep disorders, anxiety, locomotor activity and sexual behavior are regulated in much more complex ways, with a large number of different neuropeptides and their respective receptors being involved. It is also of note that the effects observed after manipulation of central CRH levels are consistent with stress-related behavioral adaptations. In this context, anxiety is certainly the best-documented phenomenon so far. Because it remains unprovable whether depression exists in rodents, it also remains open whether CRH produces depression in these animals. However, the examples selected provide compelling evidence that many cardinal symptoms characteristic of depression are most likely mediated by CRH.

4. Involvement of CRH in central neurotransmitter systems

The high density of CRH-immunoreactive fibers in the LC, which contains almost 50% of the brain norepinephrine (NE) neurons, and the recent evidence for synaptic contacts between CRH terminals and LC dendrites (van Bockstaele et al., 1996) have led to many studies exploring how hormonal, autonomic and behavioral effects of stress are co-ordinated through interactions of CRH with the LC (Valentino et al., 1993). As mentioned earlier, infusion of CRH into the LC of freely moving rats produces an increase in catecholamine activity and turnover in the frontal cortex along with increased anxiety-related behavior (Butler et al., 1990).

This is in keeping with experiments by Valentino and Webby (1988), which show that CRH administration, like acute stress, increases the firing rate of LC neurons, whereas administration of a CRH antagonist blocks this effect. Chronic stress also increases the expression of tyrosine hydroxylase, the rate-limiting enzyme of catecholamine biosynthesis. That stress-elicited CRH participates in this action as well can be demonstrated by treatment with the α -helical CRH₁₋₄₁ antagonist which effectively prevents the induction of this enzyme (Melia and Duman, 1991).

Other brain regions that are both innervated by CRH terminals (Swanson and Simmons, 1989) and also contain mRNA for CRH receptors (Chalmers et al., 1995) are the dorsal and median raphe nuclei, from where serotonergic projections to the forebrain emerge. Stress is believed to activate 5-hydroxytryptamine (5-HT) from raphe nuclei, and because CRH innervates this brain region, studies have been conducted to investigate how stress-elicited CRH and 5-HT release are linked. Singh et al. (1991) found that CRH increased the activity of tryptophan hydroxylase, the rate-limiting enzyme in the synthesis of 5-HT, in midbrain and cortex. A more recent study by Price et al. (1998) demonstrated that icv administration of CRH to freely moving rats has biphasic effects as the dosages of 0.1 and 0.3 μ g decreased the dialysate 5-HT concentrations in the lateral striatum while 3.0 μ g increased them. Because forebrain 5-HT levels are strongly affected by behavioral arousal (Rueter and Jacobs, 1996), the increase in 5-HT after very high CRH dosages may be related to behavioral activation. Linthorst et al. (1997) monitored 5-HT release by microdialysis in the hippocampus of freely moving rats and also assessed the behavioral activation by visual observation. After infusion of a low dose of CRH ($1 \mu\text{g} \cdot \mu\text{l}^{-1} \cdot \text{h}^{-1}$) for 7 days into the brain of these rats, hippocampal 5-HT levels remained unchanged. However, if CRH-treated rats were stressed by intraperitoneal injections of lipopolysaccharide, they exhibited a blunted 5-HT response (Fig. 3) and delayed onset of behavioral inhibition.

These experiments suggest that stress-elicited CRH, by acting on dorsal raphe nuclei, is capable of inhibiting 5-HT activation.

A fairly close relationship has been suggested to exist between stress-elicited CRH and γ -aminobutyric acid (GABA)-gated chloride channels. Imaki and Vale (1993) showed that benzodiazepines are able to suppress the synthesis and release of CRH. The suppressive effect of benzodiazepines on HPA activation is clinically well documented and is supported by neuroanatomical studies, which show that dense GABAergic innervation is present in hypothalamic nuclei that control anterior pituitary function. The GABA/benzodiazepine sites are also present in hippocampal and amygdala regions that are under CRH control and provide a route for neocortical effects on the hypothalamus (Swanson et al., 1983; Rich-

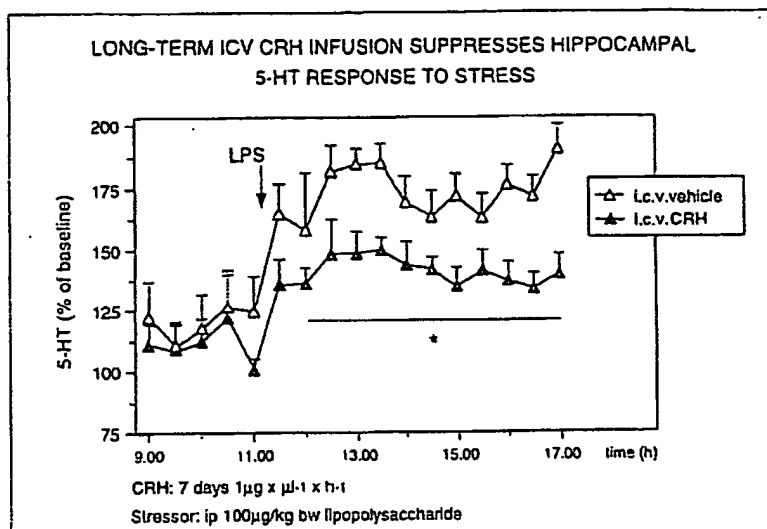


Fig. 3. Rats were intracerebroventricularly (icv) infused with either CRH ($1\mu\text{g}/\mu\text{l} \times \text{h}$) or vehicle. When both groups were stressed with lipopolysaccharide ($100\mu\text{g/kg}$ intraperitoneally), those rats treated with CRH responded with a comparatively minor serotonin release in the hippocampus, as measured by in vivo microdialysis (from: Linthorst et al., 1997).

ards et al., 1987; Sakaue et al., 1988). These CRH effects appear to be negatively controlled by GABAergic actions. The involvement of GABA-gated ion channels is also supported indirectly by studies showing that neuroactive steroids such as allopregnanolone (tetrahydroprogesterone) and THDOC are capable of suppressing CRH, which would explain their anxiolytic effects (Patchev et al., 1994b, 1997). The effect of benzodiazepines on HPA activity has also been studied by Owens et al. (1989), who showed that administration of alprazolam decreased the CRH content in the LC. More recently, Wilson et al. (1996) demonstrated that these effects are influenced by gender, which may also explain the sexual dimorphism in stress-induced corticosterone and ACTH release.

Excitatory amino acids (EAAs) have a stimulatory effect on ACTH and cortisol secretion (Jezova and Oprslova, 1992), but it is not quite clear whether EAAs act at a central level to co-regulate the synthesis and release of ACTH through specific binding sites (Meeker et al., 1994). The possibility that EAAs activate the HPA system via CRH is supported by the finding that *N*-methyl-D-aspartate (NMDA)-increased cortisol release is prevented by icv infusion of antiserum against CRH in rhesus monkeys (Reyes et al., 1990). Moreover, in vitro studies analysing the secretion of CRH from hypothalamic slices showed that EAAs, through NMDA and metabotropic receptors, but not through kainic or AMPA receptors, activate CRH secretion (Joanny et al., 1997). In contrast, other studies found no CRH release (Costa et al., 1992) or decreased CRH release (Patchev

et al., 1994a) after exposure to EAAs, which was related to methodological differences. Nevertheless, in vivo studies support a CRH-activating effect of EAAs either through direct hypothalamic actions or through afferents subserving CRH cell bodies because systemic administration of MK801 (a non-competitive antagonist of NMDA receptors) suppresses stress-induced HPA activation. The interaction between CRH and EAAs is of particular interest since EAAs, acting mainly via NMDA receptors, produce neurotoxic effects that are believed to be a primary cause of focal ischemic brain damage. Moreover, CRH is suspected of mediating neuronal damage induced by focal ischemia or NMDA receptor activation, perhaps through direct effects on neuronal activity (Aldenhoff et al., 1983; Strijbos et al., 1994). In line with this suggestion is the finding that glutamate- or ischemia-induced infarction size is decreased by co-administration of an α -helical CRH receptor antagonist. Likewise, ethanol withdrawal, which is accompanied by increased CRH release and anxiety-related behavior, is also accompanied by increased concentrations of CSF indices of EAA neurotransmission (Tsai et al., 1998), which may lead to oxidative stress and subsequent neurodegeneration (Coyle and Puttfarcken, 1993; Behl, 1998).

5. Causation of increased CRH in depression

Given that excessive CRH accounts for the well-documented HPA hyperdrive and a number of autonomic

signs and psychopathological symptoms in individuals with depression, the question of why CRH is not adequately regulated in these patients remains. The best-studied brain regions are the hippocampus and the hypothalamic PVN, where adrenalectomy was repeatedly shown to stimulate CRH biosynthesis and release to an extent similar to that seen in profound stress (Antoni, 1986; Plotsky, 1990; Dallman, 1993). Activation of CRH and subsequently of ACTH and corticosterone can also be achieved by GR antagonists and glucocorticoid synthesis inhibitors. Over time, there is increasing participation of AVP in HPA activation. This neuropeptide can also induce anxiety-related behavior (Landgraf et al., 1995). As already mentioned, similar to the human condition in depression, chronic stress in rats is associated with increased CRH and AVP co-expression and co-release from the PVN via the median eminence into the portal vessels (de Goeij et al., 1991). The effect of adrenalectomy is counteracted by exogenous corticosterone, and at low dosages only AVP synthesis is affected, indicating that AVP expression is more sensitive to corticosteroid feedback signals than CRH expression (Bradbury et al., 1994). Within the limits of neuroendocrine HPA regulation it seems clear that corticosteroids restrain CRH and AVP expression through GR activation. Because glucocorticoids are elevated in most patients with major depression, the question arises of whether there is a GR resistance in depression and if so whether this resistance is inherited or acquired.

Modell et al. (1997) performed the combined dexamethasone/CRH test in patients and controls, using increasing dosages of dexamethasone before stimulation with a fixed CRH dose. As illustrated in Fig. 4, depressed patients showed a shift of the response curve toward lower sensitivity. This effect was much less pronounced after clinical remission. Healthy subjects at genetic risk for depression also show this phenomenon as a trait (Holsboer et al., 1995b; Modell et al., 1998), suggesting the presence of GR resistance in a population with inherited susceptibility for depression. In such individuals, glucocorticoid signaling may be disturbed to a degree that does not precipitate depression under normal conditions. However, under chronic stress the impaired GR signaling may ultimately lead to cellular dysregulation that can no longer be compensated, resulting in the clinical syndrome. Work by Kendler (1998) in New Haven, U.S.A., suggests that the depressogenic effect of stressful life events is substantially greater in individuals at genetic risk for depression. Future results from the Munich Vulnerability Study will show whether those individuals who are at risk because of a high genetic load for depression and who show aberrant HPA symptoms will exhibit a manifestation of depression in later life (Lauer et al., 1998). Alternatively, the impaired GR signaling in individuals at genetic risk may render them susceptible for stressful life events. This possibility is also

in keeping with a study by Kendler and Karkowski-Shuman (1997) suggesting that genetic factors influence the kinds of stressful life events to which people expose themselves. Those with inherited premorbid HPA dysregulation due to decreased GR signaling are perhaps the ones who expose themselves to harmful life events, resulting in insufficiently restrained HPA activation, which subsequently results in depression through excessive CRH synthesis and release.

It is important to recognize that CRH can activate its own expression in the PVN. Parkes et al. (1993) injected CRH into the lateral ventricle of rats and showed that two separate immediate early genes, *c-fos* and *nur77* (also called NGFI-B), were expressed, followed by increased expression of CRH mRNA as quantified by in situ hybridization. This mechanism is perhaps life-sustaining as it keeps the organism responsive to acute stressors under conditions of chronic stress. Under such conditions, negative feedback through corticosteroids is weakened. Two examples using mouse mutants demonstrate that there is no simple reciprocal interaction between GR function and CRH expression. A transgenic mouse expressing antisense directed to GR mRNA was generated by inverting a 1.815-bp fragment of the 3'-noncoding region of GR cDNA downstream from a 2.3-kb *EcoRI/Hind III* human neurofilament promoter and inserting it into the mouse genome (Pepin et al., 1992). These mice had an exaggerated ACTH response to stress corresponding to the deficit in GR-mediated repression of POMC gene transcription. To achieve this transrepression, corticosterone must bind to intracellular GRs, which after dissociation from chaperone proteins (heat shock proteins) dimerize and bind to negative glucocorticoid response elements (GRE) in the POMC promoter. The transgene expression in these mice apparently interferes with this mechanism, resulting in an excessive ACTH response to stress (Barden et al., 1997). In contrast to the POMC gene, from which ACTH derives, expression of the CRH gene is not increased but rather is reduced in the PVN and in the external zone of the median eminence (Dijkstra et al., 1998). To understand this, it is important to know that unlike POMC expression transrepression of CRH does not involve DNA binding of ligand-activated and dimerized GRs at GREs. The suppressive effect of GRs on CRH gene expression is achieved through the interaction of activated GRs with other proteins distant from DNA-binding sites. Thus, GRs can suppress transactivation by binding to other transcription factors. Such protein-protein interactions may include binding between an activated GR and the cAMP response element-binding protein (CREB), which activates CRH gene expression in the CRH gene promoter through cAMP response elements (CRE) (Spengler et al., 1992).

Another interesting possibility is a hypothesized interaction with the orphan receptor *nur77*, which acts as a

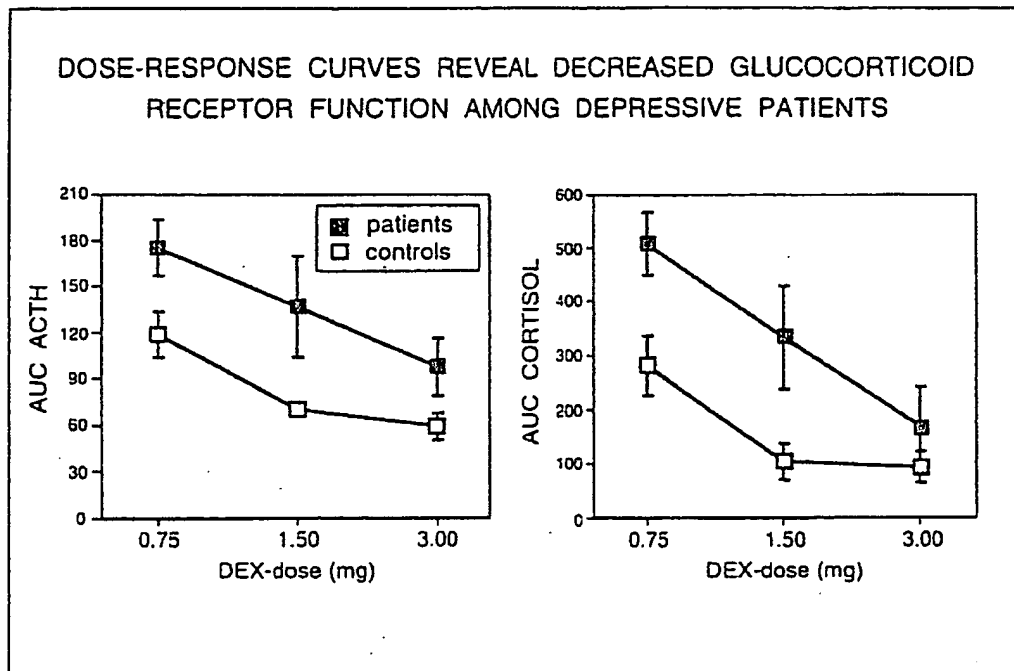


Fig. 4. Among 30 patients with major depression three different dosages of dexamethasone (DEX) (given orally at 2300 h) had a less suppressive effect on the releasable amount of ACTH and cortisol (both expressed as area under the time course curve, AUC) than among the group of matched controls, which indicates an impaired corticosteroid receptor function among depressed patients (from: Modell et al., 1997).

nuclear transcription factor that binds as an unliganded monomer to a specific DNA motif, activating expression of the CRH gene for which a *nur77* response element has been identified in the CRH gene promoter (Murphy and Conneely, 1997). Sequence analysis of the cDNA identified *nur77* as a member of the steroid receptor superfamily, and it is not unlikely that the GR heterodimerizes with *nur77* to suppress CRH gene expression because binding of *nur77* to the GR prevents CRH gene activation through the *nur77* DNA response element.

With regard to the enhanced ACTH secretion but suppressed CRH secretion in transgenic mice with impaired GR function, it appears that the genetic manipulation has preserved the GR capacity to suppress CRH gene expression through protein-protein interaction. In contrast, the transrepression requiring DNA binding of activated GR homodimers at negative GREs in the POMC promoter is apparently defunct in these mutants, resulting in enhanced ACTH release under stress. A similar dualism of HPA regulation through GR was recently observed by Reichardt et al. (1998). These authors used the finding by Heck et al. (1994) that introduction of a point mutation (arginine 458 threonine) into the amino acid sequence of the second zinc finger in the DNA-

binding domain of the GR prevents transactivation of GRE-dependent promoters. By gene targeting using the *Cre/loxP* system, Reichardt et al. (1998) created a mouse mutant in which it was possible to differentiate between transrepression and transactivation of the HPA system. Because of the mutation, these mice had elevated POMC mRNA and ACTH levels in the anterior pituitary, which reflects the need for DNA binding of GRs to exert negative feedback at the pituitary level in vivo. However, the CRH content in the median eminence was unaffected, suggesting that GR-GR dimerization is not necessary for transrepression through interaction with other transcription factors at the hypothalamic level. What still needs to be investigated is the mode of regulation of CRH in other brain structures such as the amygdala, which modulates emotional responses to stress (Gallagher and Chiba, 1996) and which has also been suggested to be implicated in conditioned fear in humans (LaBar et al., 1998). In the context of inherited impairment of corticosteroid signaling in depression, these observations are instructive insofar as they indicate that regulation of gene expression through corticosteroid receptors involves a complex nuclear assembly of transactivating factors, minor changes of which can contribute to pathology.

These transactivating factors may also serve as potential drug targets.

Whereas the studies referred to earlier focus mainly on the question of inherited dysfunction of the HPA system as a risk factor for depression, a number of animal studies have demonstrated that pre- or postnatal stressors may also affect the HPA system lifelong. Reul et al. (1994a) showed that prenatal immunostimulation of pregnant rats leads to persistent HPA hyperactivity in offspring. Other studies also showed that prenatally stressed rats have increased amygdala CRH concentrations later on and display an exaggerated hippocampal acetylcholine responsiveness following administration of CRH (Day et al., 1998).

In a series of elegant experiments, the groups of Charles Nemeroff and Paul Plotsky in Atlanta, U.S.A., studied the effects of maternal care on infant rats. They showed that those rats that had received frequent maternal care (licking, grooming) during the first 10 days of their life displayed reduced plasma ACTH and corticosteroid concentrations after stress, increased hippocampal GR mRNA and feedback sensitivity, and decreased hypothalamic CRH mRNA when they were grown-up (Liu et al., 1997). In a subsequent report the authors showed that adult rats whose mothers had licked and groomed them frequently in early infancy also had increased benzodiazepine receptor density in the amygdala and LC, increased α_2 -adrenoceptor density in the LC and decreased CRH receptor binding in the LC (Caldji et al., 1998). In contrast, rats that were postnatally traumatized by maternal deprivation alone or in combination with mild foot shocks had an increased CRH concentration in the median eminence and a decreased number of corticotropin CRH receptors (Ladd et al., 1996).

In addition to these experiments in rats, Nemeroff's group also studied primates that were exposed to adverse rearing conditions in infancy. When grown-up, those monkeys that were raised by their mothers under unpredictable foraging conditions had persistently higher CSF concentrations of CRH than those animals whose mothers had regular access to food (Fig. 5) (Coplan et al., 1996). These findings support the view that vulnerability to depression can also be acquired through early trauma such as neglect or childhood abuse. However, these important findings do not allow the conclusion that maternal deprivation per se results in long-term effects on cognition or HPA function in all affected individuals. The group led by Ronald de Kloet in Leiden, the Netherlands, studied Brown Norway rats, known for their long and healthy life span. When litters were postnatally removed from their mothers for 24 h, about half of the animals had very good cognitive abilities as adults (when aged 30 months) compared to the others who performed poorly. In those who were reared by their mothers without interruption, good and poor learning performance was normally distributed, showing that sus-

ceptibility to early trauma is influenced by individual genetic predisposition (de Kloet et al., 1998).

6. Suppressing the HPA hyperdrive with antidepressants

Serial monitoring of HPA activity and severity of depressive symptoms during treatment with antidepressants revealed that excessive HPA activity gradually decreases and that this effect precedes full clinical recovery, which suggests that normalization of stress hormone regulation is a prerequisite for clinical recovery (Holsboer, 1995a). Such a causal link between neuroendocrine signs and psychopathological symptoms is further supported by two recent observations: (1) patients who do not respond to antidepressant treatment continue to have HPA dysregulation (Holsboer et al., 1987b; Holsboer-Trachsler et al., 1994; Heuser et al., 1996) and (2) patients who are fully remitted but still have HPA dysregulation as measured with the combined dexamethasone-CRH test have a much higher risk for relapse within 6 months than patients who are fully remitted with regard to both psychopathology and neuroendocrine signs (Zobel et al., 1999).

These and many similar findings have led us to modify the description of the course of depressive illness as proposed by Kupfer (1993) in a way that takes the observed HPA dysfunction into account (Fig. 6). As the time course patterns do not appear to be influenced by the type of antidepressant, a number of studies were performed to challenge the hypothesis that antidepressants act by normalizing the HPA system. Antidepressant actions include an increase in the efficiency of glucocorticoid signaling and thus enable a more potent suppression of hormone-regulated genes either through GR interactions with other transcription factors (protein-protein interactions) as described earlier or through GR binding at DNA sequences other than the well-characterized consensus glucocorticoid response element (GRE) sequences (Phi Van et al., 1990; Malkoski et al., 1997).

The possibility that antidepressants can act by increasing GR signaling efficiency, regardless of their specific pharmacology, was suggested by Pepin et al. (1989), who showed that various antidepressants can increase GR mRNA in primary cultures of rat brain hypothalamic neurons and the amygdaloid complex. Interestingly, the tricyclic antidepressant desimipramine, which is primarily a noradrenaline reuptake inhibitor, induces activation of the GR promoter in fibroblast (LTK⁺) cells. These cells do not secrete catecholamines, which suggests that the mechanism by which tricyclics influence GR activity does not necessarily involve noradrenaline reuptake. The aforementioned mouse mutant expressing a transgene that results in functional GR impairment showed a normalized ACTH response to stress and

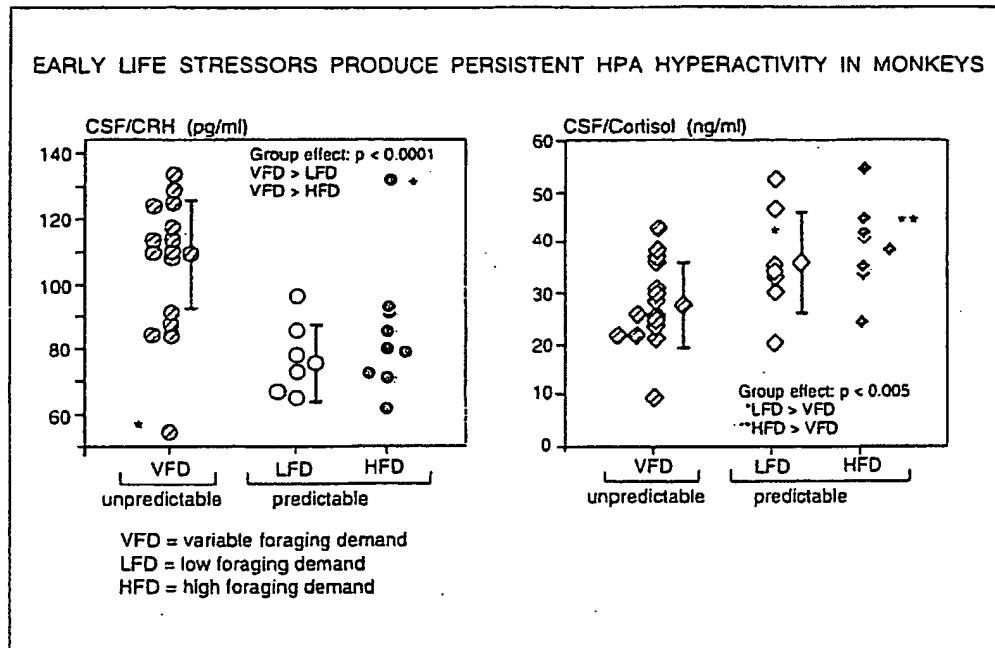


Fig. 5. Thirty bonnet macaques (*Macaca radiata*) were reared under different conditions. The HFD mothers were required to dig through wood-chip to obtain their daily food ration. During the 12-week rearing period the LFD mothers were offered abundant food items that could be picked up from the floor. The VFD mothers experienced varying conditions, either two weeks of HFD conditions or two weeks of LFD conditions. CSF samples taken a few years later from the grown-up offspring revealed persistent changes in CRH secretion, with increased levels among those grown primates that had been reared under unpredictable (VFD) foraging conditions (adapted from: Coplan et al., 1996).

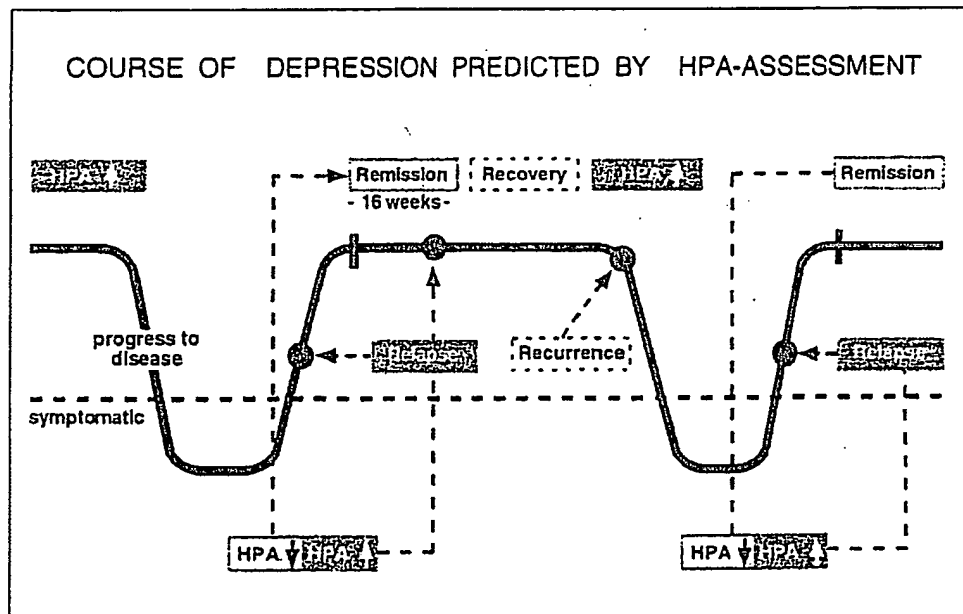
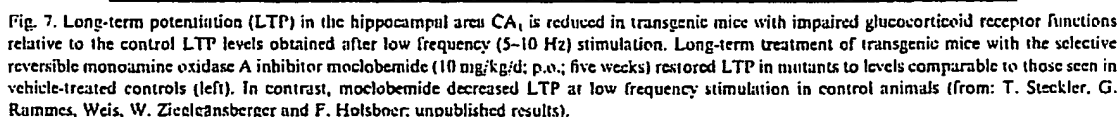


Fig. 6. Postulated relationship between the course of depression and HPA regulation.

Intrigued by the hypothesis that increased efficiency of MR signaling is a first important step in antidepressant action (Reul et al., 1993), a clinical trial was conducted

More research was directed to antidepressant actions that involve signaling through G-protein-coupled cell membrane receptors that activate kinases. Following antidepressant treatment, the acute cellular response consists in an activation of cAMP through G-protein-mediated stimulation of adenylyl cyclase. Enhancement of cAMP-stimulated protein kinase A (PKA) results in an activation of gene transcription by the cAMP/Ca²⁺-responsive element (CRE) through its cognate transcription factor, the CRE-binding protein (CREB). The CRE is a DNA motif found in promoters of many genes



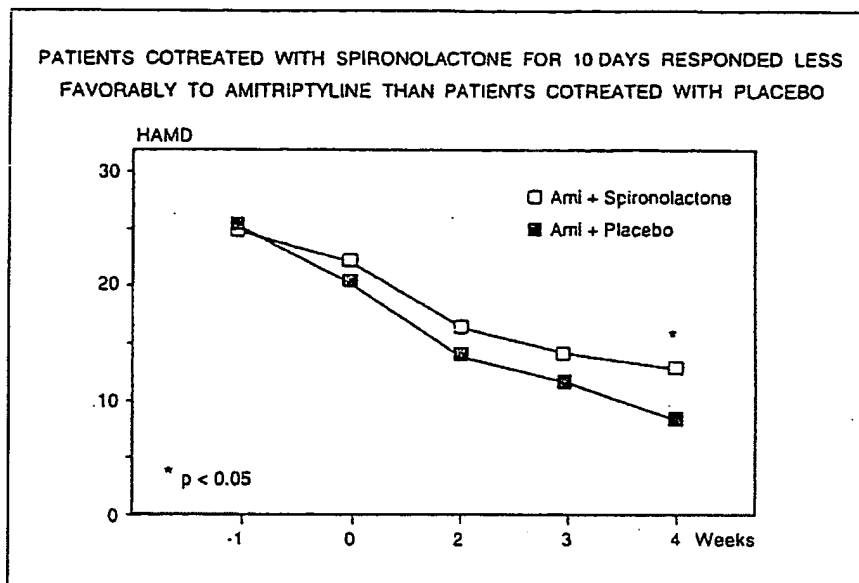


Fig. 8. Thirty patients were randomly assigned to 10 days of treatment with either 150 mg amitriptyline (Ami) plus 100 mg spironolactone (an MR antagonist) or amitriptyline plus placebo. The placebo group had a significantly better treatment outcome according to Hamilton Depression Rating Scale scores (from: W. Hundt, E. Friess, A. Ströhle, H. Reul, A. Zobel, A. Sonntag, and F. Holsboer: unpublished results).

that does not only initiate cAMP-stimulated gene transcription (Meyer and Habener 1993) but also Ca^{2+} /calmodulin-dependent protein kinase (CaM kinase)-stimulated gene transcription via an induction of membrane depolarization by Ca^{2+} inward currents that trigger the enzymatic activity. Both PKA and CaM kinase phosphorylate CREB at serine 119, which is necessary for transcriptional activation (Sheng et al. 1990). The acute effect of most antidepressants is an increase in neurotransmitters at 5-HT or norepinephrine receptors, or both, which, by activating adenylyl cyclase through G-proteins, increase the intracellular cAMP pool. Subsequently, cAMP binds to regulatory subunits of PKA, enhancing the phosphorylation of a number of PKA substrates, such as CREB, which, when phosphorylated, directs transcription of CRE-regulated genes.

After long-term administration of antidepressants, several adaptational changes occur. As first shown by Fridolin Sulser et al. (1978), prolonged exposure of the noradrenergic neuron to NA-reuptake-inhibiting antidepressants results in desensitization of β -adrenoceptors through a mechanism that has been identified only some years ago (Lefkowitz, 1993). As illustrated in Fig. 9, dissociation of the α -guanosine triphosphate (GTP) subunit from the complete $\alpha\beta\gamma$ -G-protein assembly allows the formation of a complex between the $\beta\gamma$ -dimer and the β -adrenoceptor kinase (β -ARK). This complex binds to the β -adrenoceptor and induces its phosphorylation through protein kinases in association

with β -arrestin, which terminates β -adrenoceptor-mediated signaling. This mechanism leads via decreased cAMP and subsequent decrease in PKA activation to reduced phosphorylation of substrates, such as CREB. Antidepressants also inhibit depolarization-induced Ca^{2+} influx into neuronal cells (Cai and McCaslin, 1992) and L-type Ca^{2+} currents in primary neuronal cells (Choi et al., 1992). Provided that these mechanisms also apply under pharmacotherapeutic conditions, one would expect that antidepressant-decreased Ca^{2+} influx will result in reduced phosphorylation of CaM kinase substrates, including CREB. Both antidepressant effects at the cell membrane, i.e. β -adrenoceptor stimulation and inhibition of Ca^{2+} influx, result over time in decreased CREB/CRE-directed gene transcription. These effects are most likely amplified, because three CREs within the CREB gene were identified, suggesting that the CREB gene itself is autoregulated through a CREB/CRE mechanism (Meyer and Habener, 1993).

The antidepressant-induced acute effects and long-term adaptations also have immediate and delayed effects on the HPA system, because the CRH gene expression is directed through CREB/CRE transactivation. Phi Van et al. (1990) observed stimulation of the human CRH gene promoter by cAMP in the mouse AtT 20 anterior pituitary cell line and postulated a CRE motif at position -221 base pairs, which is compatible with the finding of a cAMP-activated CRH promoter in the rat (Seasholtz et al., 1988). By progressive 5' end deletions of the human

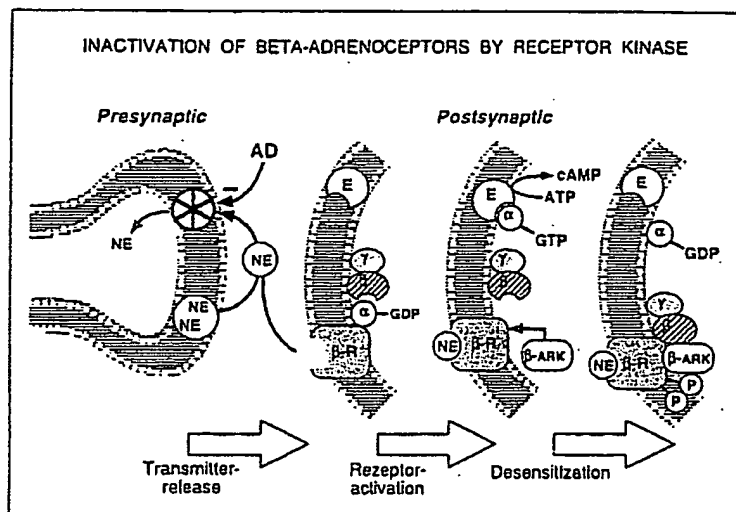


Fig. 9. Model of β -adrenoceptor 'down-regulation' through continuous ligand activation as proposed by Lefkowitz (1993). NE = norepinephrine; AD = antidepressant; β -R = β -adrenoceptor; α, β, γ = G-protein subunits; β -ARK = β -adrenoceptor kinase; GTP, GDP = Guanosine tri (di)phosphate.

CRH promoter, Spengler et al. (1992) identified a region that contained the sequence TGACGTCA at -221 base pairs, consistent with a perfect consensus motif for CRE involvement (Fig. 10). This finding explains the acute effect of antidepressants on HPA activity, which is stimulatory, as well as the long-term effects, which are suppressive (Fig. 11) (Holsboer and Barden, 1996). The suppressive effects after long-term antidepressant treatment have been well-documented by repeated administration of the dexamethasone-CRH test to patients with depression (Heuser et al., 1994; Holsboer-Trachsler et al., 1994; Zobel et al., 1999, in press) and to healthy controls (Heuser et al., 1996). Michelson et al. (1997) reported decreased plasma ACTH and cortisol responses to AVP in healthy volunteers who received imipramine at therapeutic dosages for six weeks. This finding indicates that less CRH is released endogenously after treatment and that the synergizing effect of intravenously administered AVP on ACTH secretion is consequently restricted. However, apart from this CRH-suppressive effect of antidepressants, which is well-characterized at the cellular and molecular level both in animals and humans, there are also some other adaptational responses to long-term antidepressant treatment that are of particular interest. It has been reported that chronic administration of antidepressants leads to altered binding and activation of cAMP (Nestler et al., 1989; Perez et al., 1989, 1991) and also to changes in the phosphorylation state (Racagni et al., 1992; Mori et al., 1998) and the concentrations of some PKA substrates, such as CREB (Nibuya et al., 1996). The finding of increased CREB mRNA in the rat hippocampus after three weeks of treatment with various

antidepressants has contributed to the hypothesis that antidepressants act through increases in CREB synthesis, which activate the expression of brain-derived neurotrophic factor (BDNF) in the hippocampus, thus counteracting the neurodegenerative effects of stress, which reportedly decrease BDNF (Duman et al., 1997). Although this hypothesis is appealing, it remains to be determined whether changes in constitutively expressed CREB have a functional significance of their own, or whether the phosphorylation of CREB is the only determinant event through which CREB acts on genes. Moreover, it has to be demonstrated changes in brain BDNF concentrations in the hippocampus play a causal role in depression.

In view of the findings that normalization of initial HPA hyperactivity precedes resolution of depressive symptomatology in the majority of cases, whereas continuation of HPA abnormalities is prognostically unfavorable, the question arises of how glucocorticoids interfere with the CREB/CRE-mediated gene transcription. As already mentioned, transrepression of CRH most likely occurs through protein-protein interaction, including association of ligand-activated GRs with the c-jun component of the AP1-complex and with other transcription factors that, without interaction with GRs, would be transactivators (Schüle et al., 1990). These transcription factors include *myb* and CREB, and interaction of GRs with cAMP-induced CREB/CRE-directed gene transcription has been reported particularly often. Glucocorticoid-induced regulation of the somatostatin gene, for example, depends on PKA activity and may be mediated by a GR/CREB protein-protein interaction.

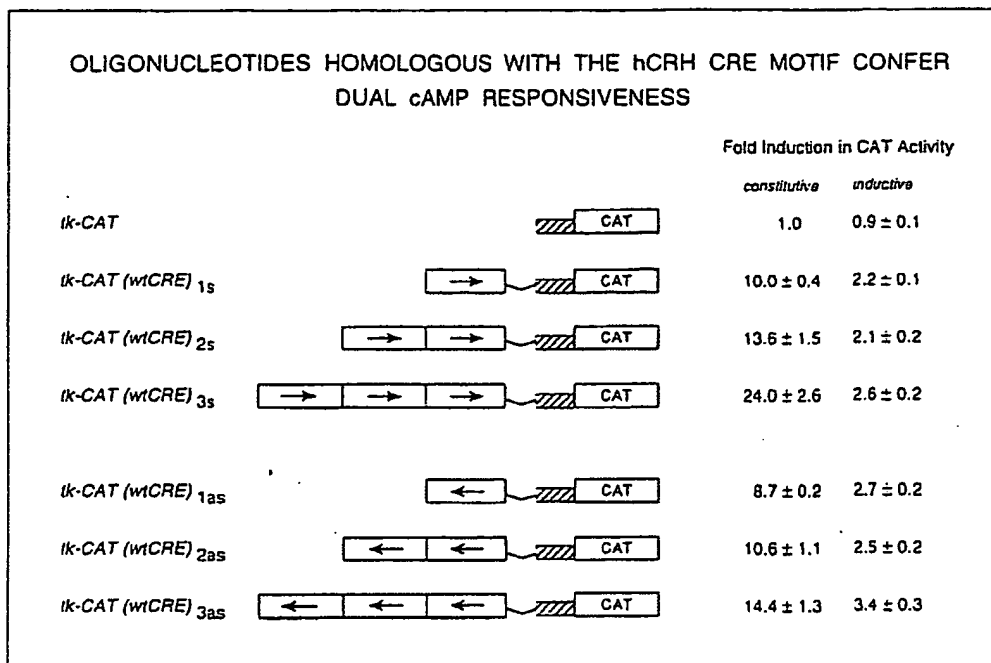


Fig. 10. An oligonucleotide (wtCRE) homologous with the sequence -238 to -216 bp of the hCRH promoter was cloned in single or multiple copies (open boxes) in forward or reverse orientation (arrows) in front of the tk promoter (hatched boxes). Constitutive expression of these constructs was referred to the parent vector (tk-CAT). Expression by treatment with 25 μ M forskolin is compared with the basal chloramphenicol acetyltransferase (CAT) activity of each construct tested. Results represent the mean \pm S.E.M. of four independent experiments expressed in terms of induction in JAR cell lines. The data show the responsiveness of the CRE in CRH gene promoter to cAMP signaling (from: Spengler et al., 1992).

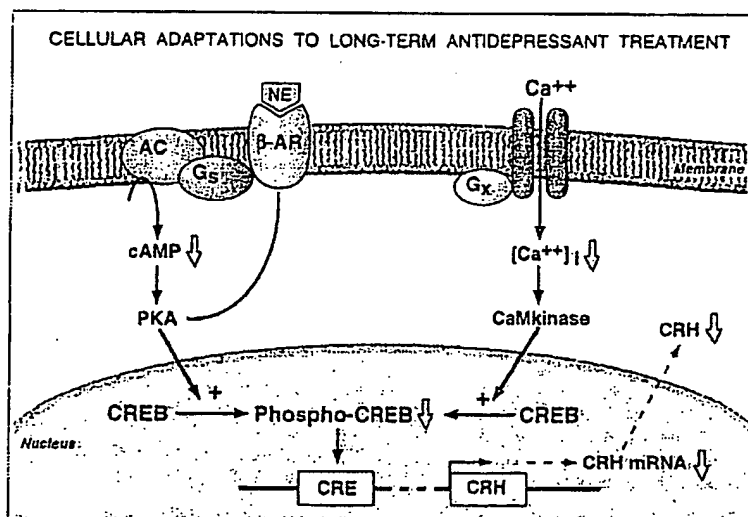


Fig. 11. Postulated effects of antidepressants leading to decreased activation (phosphorylation) of abundant cAMP-response element-binding protein (CREB) and subsequent decrease of CRH gene expression.

Similarly, GRs and cAMP amplify each other's effects on proenkephalin gene expression in C6 rat glioma cells (Joshi and Sabol, 1991), which is consistent with the mechanism suggested by the group of Ronald Evans in La Jolla, U.S.A., that PKA increases the DNA-binding activity of GR, thus upregulating corticosteroid-dependent transactivation (Rangarajan et al., 1992). Finally, investigations of P. Schmidt and colleagues (unpublished observations) recently showed that norepinephrine can enhance GR/GRE-directed gene expression through PKA-induced CREB activation. Thus, a decreased activation of both GRs and CREB after prolonged exposure to antidepressants would decrease the CREB/CRE- as well as the GR/GRE-regulated gene expression.

Of course, these effects are not uniform across cells and brain areas, and their significance for the clinical condition remains elusive. More directly related to counteracting the clinical effects of antidepressants is perhaps the finding of the group of Lefkowitz that β -adrenoceptors are transcriptionally regulated by glucocorticoids (Collins et al., 1988), which confirms earlier work by Mobley and Sulser (1980) showing that adrenoceptor agonist-induced cAMP accumulation in brain slices is controlled by corticosteroids. If this mechanism is also involved in the clinical condition of depression, the sustained activation of HPA secretion would counteract the antidepressant-induced desensitization of adrenoceptors and the subsequent decrease in intracellular signaling that regulates CREB/CRE-directed genes, including CRH.

Taken together, the reported basic research findings are consistent with the clinical observations and animal experiments which propose that antidepressants act through enhancing corticosteroid receptor function and repressing CRH gene activity. As many of the conclusions that have been drawn are weakened by the artificiality of the test systems from which they were derived, there is an obvious need to show that the *in vitro* findings are also valid at the organismic levels in animals.

7. CRH receptors

The CRH signal is mediated through functionally and regionally different cell membrane receptors. Up to now, two CRH receptors have been identified, which comprise seven putative transmembrane helices and belong to the family of G_i -protein-coupled receptors (Fig. 12). They are encoded by two distinct genes, both of which have been identified (Chalmers et al., 1996). The CRH₁ receptor was identified and cloned from a human ACTH-secreting pituitary adenoma (Chen et al., 1993), mouse pituitary (Vita et al., 1993), rat brain (Perrin et al., 1993) and human brain (Chang et al., 1993). Species homologues show 98% sequence identity over their full length of 415 amino acids. The CRH₁ receptor has five *N*-linked

glycosylation sites in the N-terminal extracellular domain and at least two potential phosphorylation sites for protein kinase C (PKC) in the first and second extracellular loop and in the C-terminal tail. Chen et al. (1993) identified an alternatively spliced form of the CRH₁ receptor in human pituitary, the functional significance of which remains to be determined. The regional and cellular distribution of CRH₁ and CRH₂ receptors is given in Table 1 and Fig. 13. It indicates that the CRH₁ receptor is very highly expressed in the cerebral cortex, the amygdala, the hippocampus, the cerebellum and the pituitary. All forms of CRH₁ receptors show a homology of about 30% to other neuropeptide receptors, including those for pituitary adenylate cyclase-activating peptide, growth hormone-releasing hormone and glucagon. The expression pattern is consistent with a role of CRH₁ receptors in mediating not only the neuroendocrine but also the behavioral and autonomic responses to stress. When expressed in stable cell lines, human/rat CRH₁ receptors show reversible, saturable and high-affinity binding of CRH, eliciting cAMP production with an EC of ≈ 1 nM.

Two different forms of the CRH₂ receptor, CRH_{2a} and CRH_{2b}, have been identified in rodents, both forms having been produced by alternative splicing (Lovenberg et al., 1995; Stenzel et al., 1995). Each has a distinct distribution and function. The CRH_{2a} receptor is found predominantly in neurons and a 411-amino acid protein that shares about 71% sequence identity with the CRH₁ receptor. The amino acid differences are located in extracellular domains, forming the ligand-binding structure, whereas the sequence identity is particularly pronounced between transmembrane domains 5 and 7 (Dautzenberg et al., 1997, 1998). These domains are involved in G_s -protein-coupled activation of the cAMP/PKA pathway, and both the CRH₁ and CRH₂ receptor forms activate this G_s -protein-coupled signaling. The localization of CRH_{2a} receptors is distinct from that of CRH₁ receptors, CRH_{2a} receptors being expressed in limited areas of the brain, including the lateral septal nucleus, the ventromedial hypothalamic nucleus and the medial amygdaloid nucleus. In rodents, the CRH_{2b} variant predominates in the brain, whereas the CRH_{2a} subtype is confined to nonneuronal structures such as the choroid plexus, cerebral blood vessels and peripheral structures in cardiac skeletal muscle, and, at lower levels, the lung and intestine (Lovenberg et al., 1995; Perrin et al., 1995).

In humans, in contrast to rodents, CRH₂ receptors exist in three different functional splice variants (α , β , γ). The CRH_{2a} and CRH_{2b} receptor variants are coexpressed in the brain and periphery (Valdenaire et al., 1997), whereas the CRH_{2c} receptor subtype seems to be expressed only in the brain, mainly in septum and hippocampus (Kostich et al., 1998). These significant differences in CRH₂ receptor expression between rodents and humans may limit extrapolations of CRH₂ receptor functions from rat to human physiology. The most potent ligand

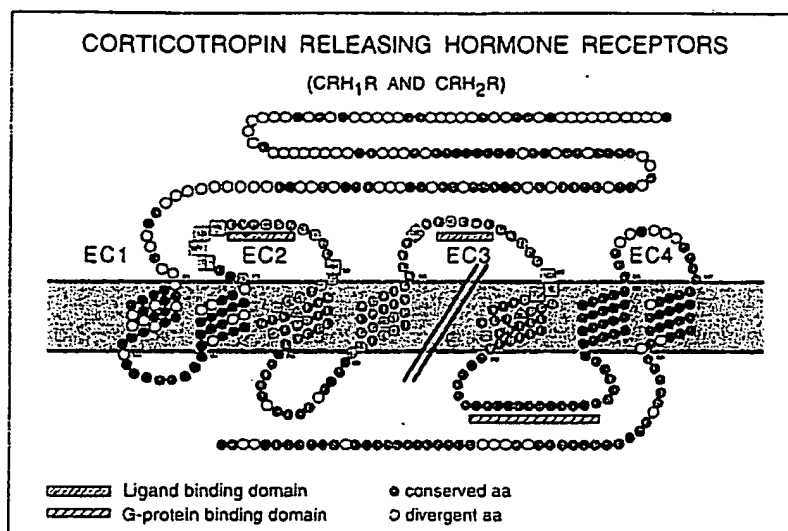


Fig. 12. CRH₁ and CRH₂ receptor structure with high conservation in the seven transmembraneous loops and the intracellular tail. The double-bar indicates that a 3.1-kb fragment of the CRH₁ receptor encoding the transmembrane domains 4–7 (including the G-protein-coupling domain and the intracellular tail) was replaced by a phosphoglycerate kinase promoter-driven neomycin cassette (Timpl et al., 1998). This intervention leaves the ligand-binding domains in place, though their binding capacity is diminished.

of CRH₂ receptors expressed in stable cell lines is urocortin, a urotensin-like peptide isolated from the Edinger-Westphal nucleus that has 45% sequence identity to CRH and has been implicated mainly in the regulation of appetite (Vaughan et al., 1995; Spina et al., 1996). The view that urocortin is the preferred mammalian ligand for central CRH₂ receptors is supported by the finding of Wang et al. (1996) that systemic administration of urocortin specifically activates neurons in the supraoptic nucleus (SON), the ventromedial hypothalamic nucleus and the magnocellular part of the hypothalamic paraventricular nucleus. Administration of urocortin induces more *fos* expression in neurons in the SON and magnocellular PVN than CRH, which is compatible with a CRH₂ receptor-mediated effect because in these structures CRH₂ receptor expression is very high. These regional and functional studies suggest that behavioral effects mediated by CRH in the structures mentioned also involve CRH₂ receptors.

Following CRH exposure, CRH₁ receptors interact with G-proteins to activate cAMP in a dose-dependent manner, which in turn activates cAMP protein kinases (Fig. 14). In addition, CRH receptors have potential PKC phosphorylation sites. PKC is activated by Ca²⁺ in conjunction with diacylglycerol, a second messenger produced by phospholipase C, which catalyzes the breakdown of phosphatidylinositol into diacylglycerol and inositol triphosphate (Duman and Nestler, 1995). According to earlier studies, CRH can modulate the Ca²⁺-dependent ion currents (Aldenhoff et al., 1983) and

intracellular Ca²⁺ increases may be dependent on CRH receptor-linked calcium channels (Takuma et al., 1994). Through these mechanisms the PKC pathway may become activated, which is important for understanding the synergy between CRH and vasopressin. The latter ACTH secretagogue, produced mainly in magnocellular neurons under basal conditions and coexpressed in parvocellular neurons under chronic stress, is a direct activator of PKC through V_{1B} receptors. Thus, the potentiating effect on activation of the POMC gene expression stems from crosstalk between PKC and cAMP-dependent kinases.

CRH₁ receptors at pituitary corticotrophs are desensitized in response to enduring agonist exposure. This desensitization is homologous as it does not affect cAMP accumulation in response to stimulation of other G_s-protein-coupled receptors. Not only long-term CRH administration but also adrenalectomy produces CRH₁ receptor desensitization at corticotrophs. The latter finding is consistent with the CRH-suppressive effect of corticosteroids on hypothalamic CRH gene expression through transrepression via protein-protein interaction (see above, and Aguilera, 1994; Rabadan-Diehl et al., 1997). A direct effect of high corticosteroid doses at pituitary cells has been reported to decrease CRH binding, but the functional significance of this effect has yet to be elucidated. According to a model proposed by the group of Lefkowitz (Fig. 9), G-protein-coupled receptors such as β -adrenoceptors are desensitized by PKA phosphorylation of residues in the third intracellular loop.

Table 1
Semi-quantitative evaluation of CRH₁ and CRH₂ receptor mRNA distribution in rat brain and pituitary gland (from Chalmers et al., 1995)

Anatomical region	mRNA abundance	
	CRH ₁	CRH ₂
Telecephalon		
Olfactory bulb:		
External granular layer	++	—
Internal granular layer	++++	++
Mitral cell layer	++++	—
Ependyma	++	+++
Accessory olfactory nucleus	++	++
Frontal cortex (superficial)	+++	—
Frontal cortex (deep)	+++	—
Cingulate cortex (superficial)	+++	—
Cingulate cortex (deep)	+++	—
Lateral septum (ventral)	+	++++
Lateral septum (intermediate)	+	++++
Medial septum	++	±
Bed nucleus of the stria terminalis (medial)	++	++
Amygdala		
Basolateral nucleus	++++	±
Medial nucleus	++++	++
Posterior cortical	+	+++
Hippocampus		
CA1	++	++
CA3/4	++++	++
Dentate gyrus	++	++
Entorhinal cortex	++	++
Diencephalon		
Hypothalamus		
Paraventricular nucleus	±	++
Supraoptic nucleus	+	+++
Lateral hypothalamus	+	+
Dorsomedial hypothalamus	+++	—
Ventromedial hypothalamic nucleus	+	++++
Medial geniculate nucleus	++	±
Mesencephalon		
Superior colliculus (superficial layer)	+++	+
Interpeduncular nucleus	++++	+++
Dorsal raphe nucleus	+	++
Caudal linear nucleus	+	+++
Red nucleus	++++	—
Pons/medulla		
Inferior colliculus	++	++
Lateral dorsal tegmental nucleus	++++	—
Locus coeruleus	—	—
Cerebellar cortex	++++	±
Pontine gray	++++	±
Motor trigeminal nucleus	++++	—
Sensory trigeminal nucleus	+++	±
Choroid plexus	—	++++
Pituitary gland		
Anterior lobe	+++	±
Intermediate lobe	+++	±
Posterior lobe	—	—

CRH₁ and CRH₂ mRNA abundance for each anatomical region was determined from optical density measurements. Density values for each parameter are presented according to their respective percentile distributions: + + + + (>75%), very dense: + + + (<75%, >50%), dense: + + (<50%, >25%), moderate: + (<25%, >0%), low: ± (<10%), scattered cells.

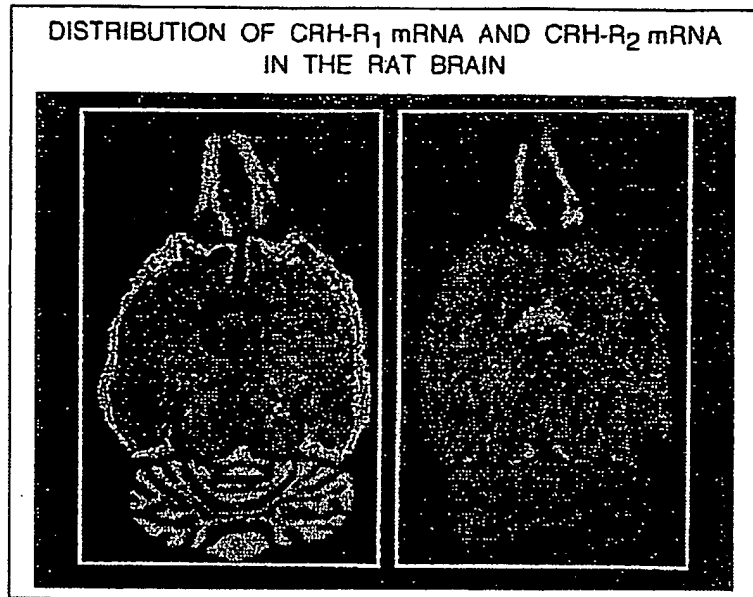


Fig. 13. CRH-R₁ and CRH-R₂ distribution as analysed by *in situ* hybridization (see also Table 1) suggests that both receptor subtypes mediate different effects by their ligands CRH and urocortin (Chalmers et al., 1995).

Whether this mechanism also applies to CRH receptors remains open, particularly because the time needed for reinstatement of receptor function is much longer than those reported for adrenoceptors (Hauger et al., 1997).

CRH₁ receptor adaptation to agonist stimulation in the brain is much less well studied. Following central CRH administration, CRH receptors were desensitized in the amygdala (Hauger and Aguilera, 1993; Hauger et al., 1993) and in the frontal cortex. In line with this is a recent study showing that acute stress elicits CRH mRNA in the central amygdala (Hsu et al., 1998), whereas long-term stress or CRH administration upregulates CRH receptors in the hippocampus, hypothalamic PVN and SON (Luo et al., 1994; Iredale et al., 1997). Down-regulation of CRH receptors in the amygdala and frontal cortex after chronic unpredictable stress may be a consequence of elevated CRH secretion under these conditions. In contrast, CRH₁ receptor mRNA was found to be increased in the hippocampus and hypothalamic PVN in response to unpredictable stress (Iredale et al., 1996). Similarly, an immune challenge and immobilization stress were reported to enhance CRH₁ receptor mRNA, which, provided that translation into receptor protein occurs, leads to enhanced CRH responsiveness in these brain regions under stress (Rivest et al., 1995). The mechanism underlying the CRH-induced upregulation of CRH₁ receptors in the hippocampus and hypothalamus may represent a feed-forward loop that maintains the organism's capacity to respond to an acute stressor under con-

ditions of chronic stress. Elucidation of the mechanism by which CRH upregulates or downregulates CRH receptors in the brain requires characterization of the promoter region of the CRH₁ receptor gene and its cell-specific regulation.

8. Preventing CRH actions by blocking its receptors

Given the evidence that the neuropeptide CRH, when hypersecreted continuously in rats, produces numerous behavioral changes resembling the cardinal symptoms of depression and anxiety, the most straightforward therapeutic strategy is a blockade of its action by CRH receptor antagonists. The first CRH receptor antagonist described (Rivier et al., 1984) was the α -helical CRH₆₋₄₁ peptide, an N-terminus-shortened analog of human/rat CRH. This molecule proved to be a competitive inhibitor of CRH-elicited ACTH secretion in pituitary cells and has been subsequently studied in a large number of behavioral experiments in rats. These studies, mainly conducted in the laboratory of George Koob in La Jolla, U.S.A., consistently showed that α -helical CRH₆₋₄₁ suppresses behavioral and neuroendocrine responses to CRH administration (Britton et al., 1986; Heinrichs et al., 1992; Koob et al., 1993), and to various emotional stressors (Heinrichs et al., 1994; Korte et al., 1994). The α -helical CRH₆₋₄₁ peptide has also been administered to humans and has been shown to block pituitary CRH

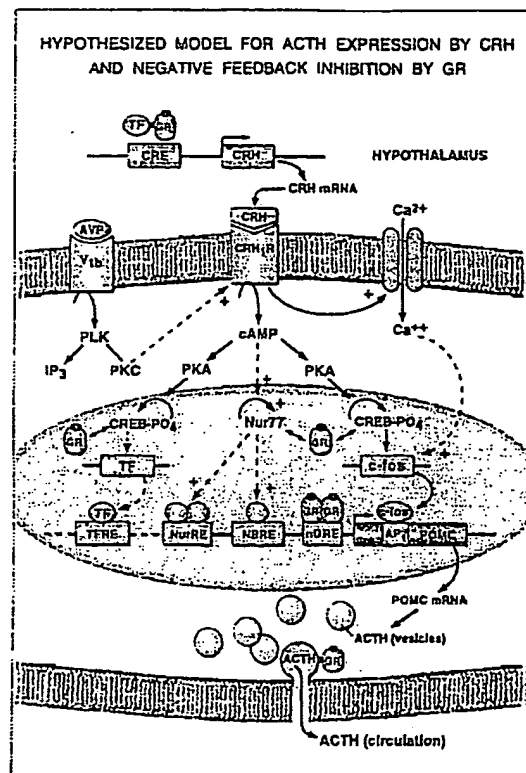


Fig. 14. This scheme explains how ACTH and corticosteroid secretion are maintained in the absence of a functional CRH₁ receptor through activation of the POMC gene via orphan receptors (*nur77*) which trans-activate via response elements (NurRE, NBRE) that require either *nur77* monomers or dimers. Other secretagogues released from the hypothalamus may also activate POMC gene expression through non-CRH₁ receptor-mediated processes. Activation of CRH₁ receptors through CRH results in enhanced *nur77* activation and a further activation of a variety of transcription factors (TF, c-fos) which amplify each other in enhancing POMC gene expression. Counterregulation requires dimerization of ligand-activated glucocorticoid receptors and subsequent DNA binding at negative response elements (nGRE).

receptors, as evidenced by decreased ACTH and cortisol secretion (Baran et al., 1996a). The peptide was given as an intravenous infusion, and the absence of anxiolytic effects was attributed to its inability to cross the blood-brain barrier. However, the individuals studied were young healthy controls without any signs of psychopathology. The anxiolytic effects of central CRH receptor blockade are to be expected only if CRH secretion is elevated in pathological conditions during severe stress, which is never the case in healthy individuals at rest. Nevertheless, a possible central effect of peripherally administered α -helical CRH₄₋₄₁ peptide cannot be excluded as peripherally administered neuropeptides, provided the peptide is given in a pulsatile mode, can

have distinct CNS effects, for example on sleep (Steiger and Holsboer, 1997).

One major disadvantage of α -helical CRH₄₋₄₁ is that it showed CRH-like weak intrinsic agonistic ('stress-like') activity in some but not all in vitro and in vivo studies (Baldwin et al., 1991; Rainnie et al., 1992; Wiersma et al., 1995). A new CRH-derived peptide in which the N-terminus was shortened in addition to several amino acid substitutions, D-Phe¹², Nle^{21,32}, (zMeLeu²⁷) CRH₁₂₋₄₁ (abbreviated D-Phe CRH₁₂₋₄₁), was introduced by Menzaghi et al. (1994) and shown to block CRH-induced behavioral responses such as locomotor activation five times more potently than α -helical CRH₄₋₄₁, which is consistent with the in vitro data demonstrating that D-Phe CRH₁₂₋₄₁ was eight times more potent in preventing CRH-induced ACTH secretion. In addition to its higher potency, D-Phe CRH₁₂₋₄₁ proved to lack intrinsic agonistic effects (Menzaghi et al., 1994). In the same laboratory D-Phe CRH₁₂₋₄₁ was used to test whether the anxiogenic-like effect of cannabinoid receptor stimulation by the synthetic agonist HU 210 could be blocked by CRH antagonists (Rodriguez de Fonseca et al., 1996). Cannabinoids activate the HPA system, and the associated anxiogenic effects are likely to be mediated by CRH receptors as D-Phe CRH₁₂₋₄₁ is capable of attenuating the behavioral effect. Systemic cocaine administration produced a conditional saccharine aversion, which was dose-dependently potentiated by central administration of D-Phe CRH₁₂₋₄₁, implicating CRH activation in cocaine-related motivational states, too (Rodriguez de Fonseca et al., 1996, 1997).

Neither α -helical CRH₄₋₄₁ nor D-Phe CRH₁₂₋₄₁ possesses the property to selectively bind to different CRH-receptor subtypes, but a drug with a potential for clinical efficacy needs to be targeted against specific CRH receptors in order to limit side effects. One strategy for delineating which CRH receptor subtype mediates CRH-induced psychopathology consists in using antisense probes. Liebsch et al. (1995) infused an antisense ODN corresponding to the CRH₁ receptor mRNA bilaterally into the central amygdala of rats for four days prior to subjecting the animals to social defeat and subsequent testing of anxiety-related behavior. As illustrated in Fig. 15, ODNs corresponding to the CRH₁-receptor mRNA had anxiolytic effects in rats. These findings were compatible with those obtained in studies using ODNs corresponding to CRH mRNA (Skutella et al., 1994a,b) and in a study by Swiergiel et al. (1993) showing that stress-induced behavior is attenuated after CRH receptor antagonist infusion into the central amygdala. In a subsequent study, Skutella et al. (1998) investigated extensively the effects of an antisense probe corresponding to CRH₁ receptor mRNA in vitro and in vivo. The antisense probe reduced CRH binding and function, as measured by ACTH secretion, in primary rat anterior pituitary cells and in clonal mouse pituitary tumor cells (AtT 20), and

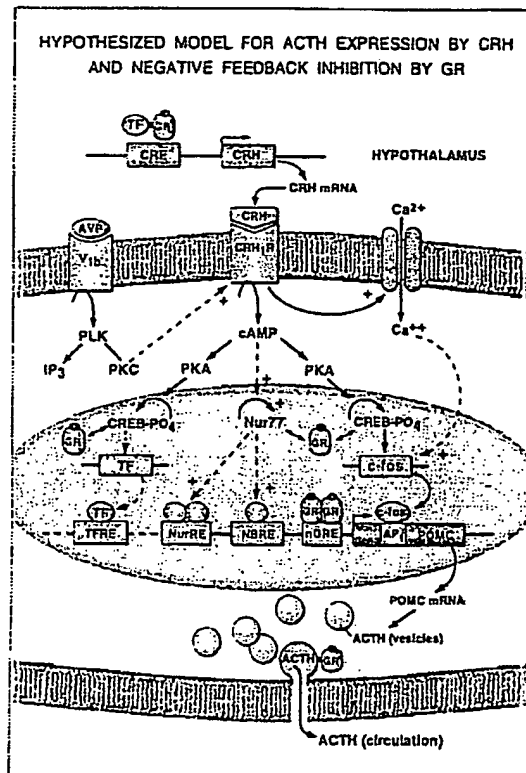


Fig. 14. This scheme explains how ACTH and corticosteroid secretion are maintained in the absence of a functional CRH₁ receptor through activation of the POMC gene via orphan receptors (*nur77*) which trans-activate via response elements (NurRE, NBRE) that require either *nur77* monomers or dimers. Other secretagogues released from the hypothalamus may also activate POMC gene expression through non-CRH₁ receptor-mediated processes. Activation of CRH₁ receptors through CRH results in enhanced *nur77* activation and a further activation of a variety of transcription factors (TF, c-fos) which amplify each other in enhancing POMC gene expression. Counterregulation requires dimerization of ligand-activated glucocorticoid receptors and subsequent DNA binding at negative response elements (nGRE).

receptors, as evidenced by decreased ACTH and cortisol secretion (Baram et al., 1996a). The peptide was given as an intravenous infusion, and the absence of anxiolytic effects was attributed to its inability to cross the blood-brain barrier. However, the individuals studied were young healthy controls without any signs of psychopathology. The anxiolytic effects of central CRH receptor blockade are to be expected only if CRH secretion is elevated in pathological conditions during severe stress, which is never the case in healthy individuals at rest. Nevertheless, a possible central effect of peripherally administered α -helical CRH₉₋₄₁ peptide cannot be excluded as peripherally administered neuropeptides, provided the peptide is given in a pulsatile mode, can

have distinct CNS effects, for example on sleep (Steiger and Holsboer, 1997).

One major disadvantage of α -helical CRH₉₋₄₁ is that it showed CRH-like weak intrinsic agonistic ('stress-like') activity in some but not all in vitro and in vivo studies (Baldwin et al., 1991; Rainnie et al., 1992; Wiersma et al., 1995). A new CRH-derived peptide in which the N-terminus was shortened in addition to several amino acid substitutions, D-Phe¹², Nle^{21,38}, (α MeLeu³⁷) CRH₁₂₋₄₁ (abbreviated D-Phe CRH₁₂₋₄₁), was introduced by Menzaghi et al. (1994) and shown to block CRH-induced behavioral responses such as locomotor activation five times more potently than α -helical CRH₉₋₄₁, which is consistent with the in vitro data demonstrating that D-Phe CRH₁₂₋₄₁ was eight times more potent in preventing CRH-induced ACTH secretion. In addition to its higher potency, D-Phe CRH₁₂₋₄₁ proved to lack intrinsic agonistic effects (Menzaghi et al., 1994). In the same laboratory D-Phe CRH₁₂₋₄₁ was used to test whether the anxiogenic-like effect of cannabinoid receptor stimulation by the synthetic agonist HU 210 could be blocked by CRH antagonists (Rodriguez de Fonseca et al., 1996). Cannabinoids activate the HPA system, and the associated anxiogenic effects are likely to be mediated by CRH receptors as D-Phe CRH₁₂₋₄₁ is capable of attenuating the behavioral effect. Systemic cocaine administration produced a conditional saccharine aversion, which was dose-dependently potentiated by central administration of D-Phe CRH₁₂₋₄₁, implicating CRH activation in cocaine-related motivational states, too (Rodriguez de Fonseca et al., 1996, 1997).

Neither α -helical CRH₉₋₄₁ nor D-Phe CRH₁₂₋₄₁ possesses the property to selectively bind to different CRH-receptor subtypes, but a drug with a potential for clinical efficacy needs to be targeted against specific CRH receptors in order to limit side effects. One strategy for delineating which CRH receptor subtype mediates CRH-induced psychopathology consists in using antisense probes. Liebsch et al. (1995) infused an antisense ODN corresponding to the CRH₁ receptor mRNA bilaterally into the central amygdala of rats for four days prior to subjecting the animals to social defeat and subsequent testing of anxiety-related behavior. As illustrated in Fig. 15. ODNs corresponding to the CRH₁-receptor mRNA had anxiolytic effects in rats. These findings were compatible with those obtained in studies using ODNs corresponding to CRH mRNA (Skutella et al., 1994a,b) and in a study by Swiergiel et al. (1993) showing that stress-induced behavior is attenuated after CRH receptor antagonist infusion into the central amygdala. In a subsequent study, Skutella et al. (1998) investigated extensively the effects of an antisense probe corresponding to CRH₁ receptor mRNA in vitro and in vivo. The antisense probe reduced CRH binding and function, as measured by ACTH secretion, in primary rat anterior pituitary cells and in clonal mouse pituitary tumor cells (AtT 20), and

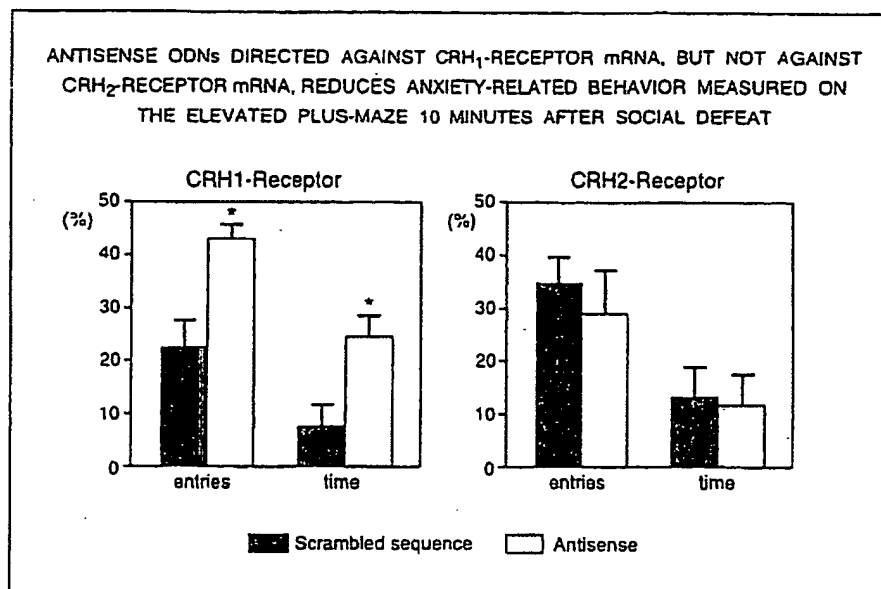


Fig. 15. In the elevated plus-maze test socially defeated rats show less anxiety-related behavior (more entries and more time spent on open arms) if chronically pretreated with an antisense probe directed against the CRH₁, but not against the CRH₂, receptor mRNA (see Table 2, from: Liebsch et al., 1999, in press).

provided evidence that this action is associated with cytoplasmic uptake of the probe.

Intracerebroventricular infusions of antisense, sense and mismatch probes to rats confirmed that inhibition of CRH₁ receptor mRNA translation into CRH₁ receptor protein reduces CRH-elicited anxiety-related behavior in rats. This effect was associated with decreased CRH binding in the hypothalamus and cortex (Skutella et al., 1998). The localization of CRH₂ receptors suggests that these receptors are also involved in the mediation of CRH-induced behavioral changes. This notion is supported by a study by Moreau et al. (1997), who showed that urocortin, which acts primarily through CRH₂ receptors, is an anxiogenic neuropeptide that also has an anorexic effect. Therefore, Liebsch et al. (1999, in press) compared the effects of antisense probes directed against CRH₁ and CRH₂ receptors. When these probes were infused chronically into the lateral ventricle of rats, partial loss of function of CRH₁ and CRH₂ receptors produced distinct behavioral effects (Table 2). As expected, there was an anxiolytic effect in animals treated with CRH₁ receptor antisense ODN, whereas no such effect was observed in those treated with CRH₂ receptor antisense ODN, thus confirming findings obtained by Heinrichs et al. (1997). However, the CRH₂ receptor antisense treatment increased immobility in a forced swim test, which suggests that the CRH₂ receptor plays a role in stress-coping behavior.

According to a widespread interpretation of the forced

swim test, (also known as the Porsolt test), reduced immobility is believed to be indicative of an antidepressive potential of the applied drug. However, this is unlikely in the context of CRH receptors and has been shown previously to be unlikely in the context of moclobemide-treated transgenic mice with impaired GR function (Montkowski et al., 1995). The studies with peptides that antagonize both of the CRH receptors and antisense probes that selectively reduce CRH receptor subtype levels indicate that CRH₁ receptors are the primary target at which selective nonpeptide compounds designed to treat anxiety and stress-related disorders should be directed.

Several drug companies have taken up this concept and employed high-speed screening of compound libraries yielding lead compounds that after chemical modifications have fulfilled the criteria for specific CRH₁ receptor antagonists. Most of these studies are unpublished for patent reasons, and therefore this report is limited to compounds the companies do not plan to take up into clinical development programs.

Schulz et al. (1996) reported that a pyrrolo[2,3-d]pyrimidine derivative, CP-154,526, produced by Pfizer Inc., binds selectively to the CRH₁ receptor subtype (Fig. 16). This compound readily enters the CNS after peripheral administration. Using increases in acoustic startle produced by icv CRH as an indicator of a CRH-elicited increase in fear and anxiety, the group found that CP-154,526 blocked the CRH effects completely. Firing

Table 2
Effect of CRH₁ and CRH₂ receptor knockdown

Test	Day	CRH ₁ receptor	CRH ₂ receptor
Social discrimination (olfactory memory)	3	no effect	no effect
Elevated plus-maze (anxiety)	4	anxiolytic	no effect
Open field (locomotor activity)	4	no effect	no effect
Forced swim test (stress coping)	5/6	no effect	increased immobility

Antisense oligodeoxynucleotides were administered intracerebroventricularly through osmotic minipumps at a rate of 5 µg/hr to male Wistar rats for seven days. Under these experimental conditions only CRH₁ receptor decrease had anxiolytic effects (Liebsch et al., 1999, in press).

STRUCTURE OF THE PYRROLO [2,3-d] PYRIMIDINE
CP-154,526

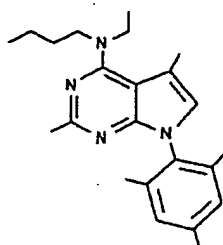


Fig. 16. High speed screening of compound libraries with a radioligand binding assay led to the discovery of a low affinity lead compound, which after several chemical modifications resulted in the discovery of CP-154,526 (butyl-[2,5-dimethyl-7-(2,4,6 trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine-4-yl]ethylamine), which has high affinities to cerebral cortical ($K_i = 5.7$ nM) and pituitary ($K_i = 1.4$ nM) membranes of rats (Schulz et al., 1996).

activity of the LC is a rapid response to anxiogenic exposures, activating noradrenaline release in many brain areas. As mentioned earlier, CRH activates the firing rate of the LC when directly injected or when administered icv, which is consistent with the presence of CRH-immunoreactive fibers and intersynaptic connections in this brainstem nucleus. When CP-154,526 is administered orally prior to CRH administration, the CRH-induced firing can be attenuated in a dose-dependent fashion. Lundkvist et al. (1996) showed anxiolytic effects of CP-154,526 employing the elevated plus-maze test. In an extensive study, Griebel et al. (1998) administered a large battery of behavioral tests to rats and mice and compared the anxiolytic effects of CP-154,526 with those of buspirone (a partial 5-HT_{1A} agonist) and diazepam. The overall conclusion was that this nonpeptide CRH₁ receptor antagonist has anxiolytic effects in stressed rodents. Because anxiety and exposure to stress are thought to contribute to drug-seeking behavior in humans and experimental animals, Shaham et al. (1998) performed a study with CP-154,526 in which they documented that

this drug can prevent the stress-induced reinstatement of cocaine- and heroin-taking in rodents in which these behaviors have been extinguished.

Because of the potential role of CRH in precipitating and maintaining depression, a preliminary report by Mansbach et al. (1997) is of note, in which it is postulated that CP-154,526 has antidepressant effects in rats that have been exposed to a series of inescapable foot shocks and then tested in a shock-escape test. Animals that are exposed to inescapable shocks perform poorly in the shock-escape procedure because of 'learned helplessness' (Wilner, 1984). This procedure is frequently used as an animal model of depression, and it has been shown that the acquisition deficits that develop in response to uncontrollable shock exposure, for instance in the form of such inability to escape from noxious stimuli, can be reversed by antidepressants (Wilner, 1984). When CP-154,526 was given prior to the escape test, it reversed the animals' deficit in perceiving and using the possibility to escape dose-dependently and much faster than imipramine, which needs to be administered repeatedly to achieve the same effects. Another pyrrolo[2,3-d]pyrimidine derivative (NBI 27914) was synthesized by Chen et al. (1996). This compound binds specifically to CRH₁ receptors and suppresses CRH-induced ACTH release in vitro and in vivo (Webster et al., 1996). CRH administration leads to seizures that originate in the amygdala and spread to the hippocampus and other limbic structures. These seizures occur at dosages that do not activate the HPA system (Baram et al., 1992) and are prevented by nonselective CRH receptor antagonists, but not by glutamate receptor antagonists (Baram et al., 1996b). In rat pups pretreated with NBI 27914, the duration of CRH-induced seizures can be shortened in a dose-dependent fashion (Baram et al., 1997). Other CRH₁ receptor-specific pyrrolo[2,3-d]pyrimidine derivatives also have anxiolytic effects in the rat line bred for high anxiety by Rainer Landgraf in Munich, which supports the view that these substances comprise a new class of compounds with a remarkable potential for clinical use in pathological anxiety and other stress related disorders (M. Keck and colleagues, unpublished data).

9. Mice lacking a functional CRH₁ receptor

A further set of experiments suited to complement the studies with antisense probes and antagonists targeted to the CRH₁ receptor uses mice with deficient CRH₁ receptors due to homologous recombination in embryonic stem cells. By deleting the coding sequences of the transmembrane regions V, VI and VII, including the G-protein coupling domain and the intracellular cytoplasmic tail (Fig. 12), a research team in Munich, led by Wolfgang Wurst, generated a mouse with a truncated protein instead of a functional CRH₁ receptor and hence unable to activate cAMP in response to CRH (Timpl et al., 1998). In cultured pituitary cells obtained from heterozygous mutants and homozygous mutants CRH evoked a decreased ACTH response, whereas forskolin, which directly activates the catalytic subunit of adenylyl cyclase, evoked a much more pronounced activation of ACTH (Fig. 17). In contrast to wild-type mice, in homozygous and heterozygous mutants CRH did not elicit a marked cAMP increase, which indicates that the mutations specifically impaired CRH/CRH₁ receptor signaling. Slightly different results emerged from *in vivo* studies, in which the basal plasma ACTH concentrations of homozygous and heterozygous mutants and wild-type mice proved to be indistinguishable, irrespective of gender (Fig. 18).

This discrepancy can be attributed to the presence of other ACTH secretagogues under *in vivo* conditions, where CRH₁ receptor-independent activation of the POMC gene, e.g., through *nur77* (Drouin et al., 1998), also occurs to maintain basal activity (Fig. 14). Under

stress conditions such as the forced swim test, plasma ACTH levels rise steeply in wild-type and heterozygous mice, whereas homozygous null mutants show decreased plasma ACTH levels. Similarly, plasma corticosterone concentrations are decreased in mice with CRH₁ receptor deficiency (Fig. 18). When CRH is administered to rodents or when a mouse overexpresses CRH through a transgene insertion, these animals display reduced exploratory behavior and increased anxiety (Stenzel-Poore et al., 1992, 1994; Koob et al., 1994). When tested in an open field test or in a light-dark box, mice lacking CRH₁ receptor function showed less anxiety-related behavior. These findings are in agreement with the histological and immunohistochemical data obtained from these mice (Figs 19, 20). No morphological changes could be detected in any of the brain areas investigated and no changes in other proteins involved in HPA regulation, including CRH₂ receptors, CRH-binding protein, MRs, GRs, and brain-derived neurotrophic factor, were observed. The only changes seen were elevations of CRH in the PVN, hippocampus, amygdala and cerebral cortex, which can be interpreted as adaptive responses to CRH₁ receptor deficiency.

Similar results were obtained by Smith et al. (1998), who generated a CRH₁ receptor-deficient mouse by replacing the last 12 amino acids of the first extracellular domain through the fourth transmembrane domain with a neomycin-resistant gene cassette, which resulted in a non-functional CRH₁ receptor protein. These mice were tested by using the dark-light emergence task and the elevated plus-maze test, in both of which they displayed markedly reduced anxiety-related behavior. The investigators stud-

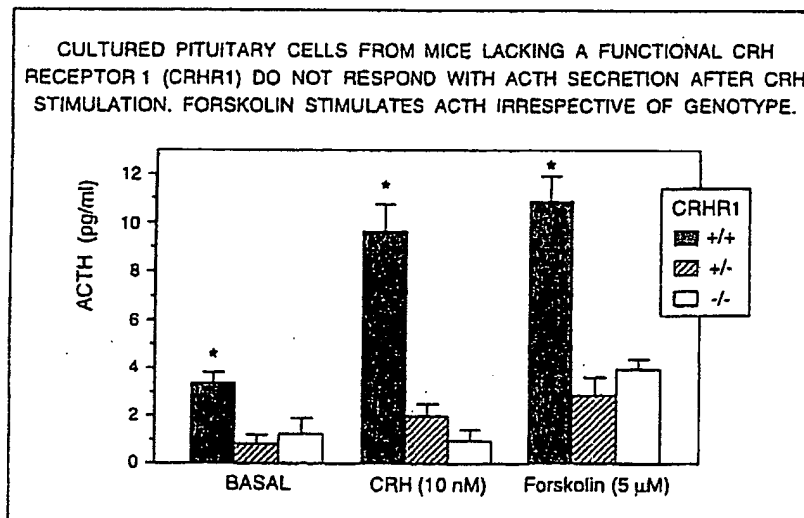


Fig. 17. In a mouse mutant that lacks a functional CRH₁ receptor ACTH release from cultured corticotrophs is decreased after CRH stimulation (adapted from Timpl et al., 1998).

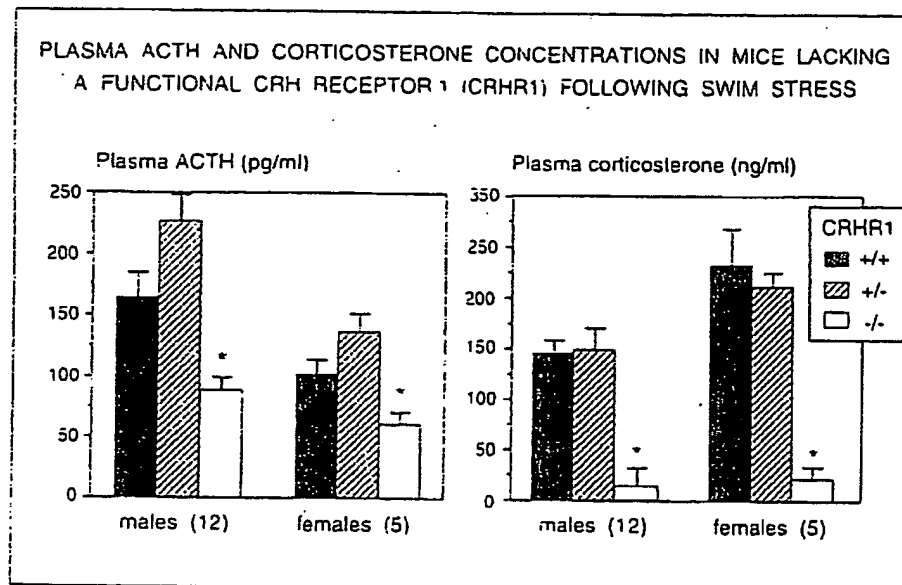


Fig. 18. In vivo studies showed that the plasma ACTH levels of homozygous and heterozygous CRH₁ receptor knockout mice and wild-type controls were indistinguishable. After forced swim stress, plasma ACTH concentrations were significantly increased in wild-type and heterozygous male and female mice, but not in homozygous mutants. Plasma corticosterone responses were also much lower in CRH₁ receptor-deficient mutants (adapted from: Timpi et al., 1998).

ied the adrenocortical changes in the CRH₁ receptor null mutants extensively and concluded that adrenal deficiency in these mice is due to decreased plasma ACTH concentrations during neonatal life (Smith et al., 1998).

One of the most severe stressors in rodents is withdrawal from alcohol, which results in excessive activation of the HPA system and strongly increased anxiety. In rodents, and most likely also in humans under clinical conditions, both phenomena are related to increased release of CRH from central neurons (von Bardeleben et al., 1989; Rassnick et al., 1993). CRH₁ receptor mutants and wild-type mice were subjected to a forced alcohol drinking procedure and were subsequently tested under withdrawal conditions (Timpi et al., 1998). In these investigations a CRH overactivity, mediated via CRH₁ receptors, could be demonstrated. When the animals were tested in the light-dark box, the reduced latency to enter the lit compartment and the increased time spent there served as indicators for a decrease in the rank order of displayed anxiety: homozygous < heterozygous < wild-type, which is consistent with a gene/dosage effect of the CRH₁ receptor-mediating anxiety-related behavior during alcohol withdrawal (Fig. 21).

10. CRH-binding protein

Further fine-tuning of the HPA system is accomplished by the presence of CRH-binding protein (CRH-BP) (Pot-

ter et al., 1991). This protein binds CRH with high affinity, and as it is localized in the pituitary, it can diminish the production and release of ACTH. CRH-BP is also broadly distributed in the brain, where it colocalizes in some areas with CRH and its receptors, a finding that supports its role as a modulator of CRH-induced behavioral and autonomic effects. In cases where the CRH:CRH-BP ratio is increased, activation of CRH receptor-mediated effects occurs (Potter et al., 1992, 1994). If the amount of CRH but not of CRH-BP is decreased, CRH receptor activation is attenuated. The latter condition is believed to be present in patients with Alzheimer's disease, in whom CRH has been demonstrated to be decreased in cortical areas and cerebrospinal fluid (Bissette et al., 1985; Pomara et al., 1989). In these patients CRH receptors were upregulated and CRH-BP was unchanged (De Souza et al., 1986). A study by Potter et al. (1992) suggested that 60-90% of the total CRH present in the human brain is bound to CRH-BP and thus prevented from exerting biological effects on CRH receptors. It is of note that CRH-BP binds CRH with a 10-fold higher affinity than the CRH₁ and the CRH₂ receptor (Chang et al., 1993; Perrin et al., 1995). The highest concentrations of CRH-BP were found in the hypothalamus, the central amygdala and the hippocampus. The decrease in free CRH and CRH/CRH-BP in patients with Alzheimer's disease was hypothesized to account for the cognitive decline in these patients because CRH was shown to have cognition-enhancing

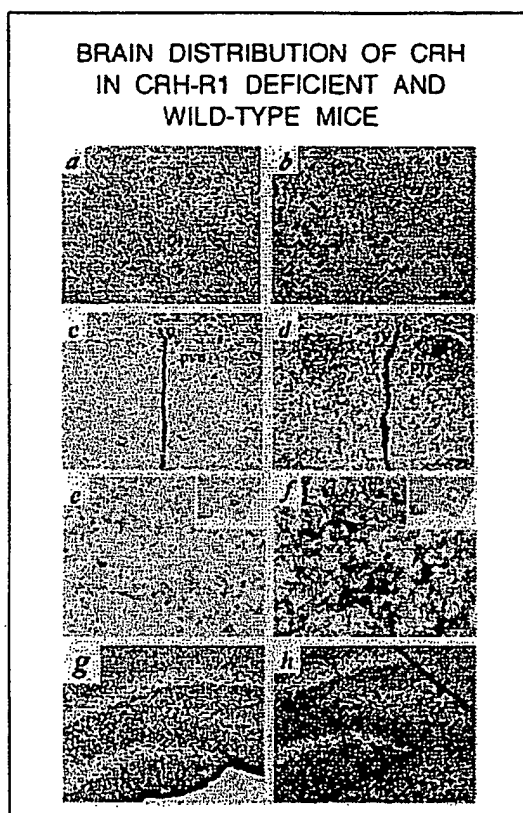


Fig. 19. Immunohistochemical analysis of CRH distribution in wild-type mice (left) and mutants with CRH receptor deficiency (right). In the mutants, CRH is increased in the perikarya and fibers within layer V of the neocortex (a,b), in the paraventricular nucleus (PVN) of the hypothalamus (c,d), in the central amygdala (e,f), and in the polymorphic layer of the hippocampal dentate gyrus (PoDG). These data indicate that via short-loop feedback CRH expression is enhanced in the absence of functional CRH receptors (from: Timpl et al., 1998; A. Kresse and M. Müller, unpublished results).

effects. Consequently, Behan et al. (1995a) administered a peptide (CRH₆₋₃₃) that displaces CRH from CRH-BP and showed that memory and learning were improved. They also showed that it is possible to dissociate the cognition-enhancing effects of CRH from its anxiogenic arousal effects. Behan et al. (1995a) attributed this to the relative enrichment of CRH-BP in the hippocampus and the cerebral cortex, brain regions that are involved more in cognitive than in emotional responses to CRH. The same group investigated brains of Alzheimer patients and confirmed that CRH-BP is capable of limiting the biological actions of CRH in various brain regions, particularly the cerebral cortex (Behan et al., 1997). Because of the high concentration of CRH in the hippocampus and the stimulatory actions of CRH on hippocampal

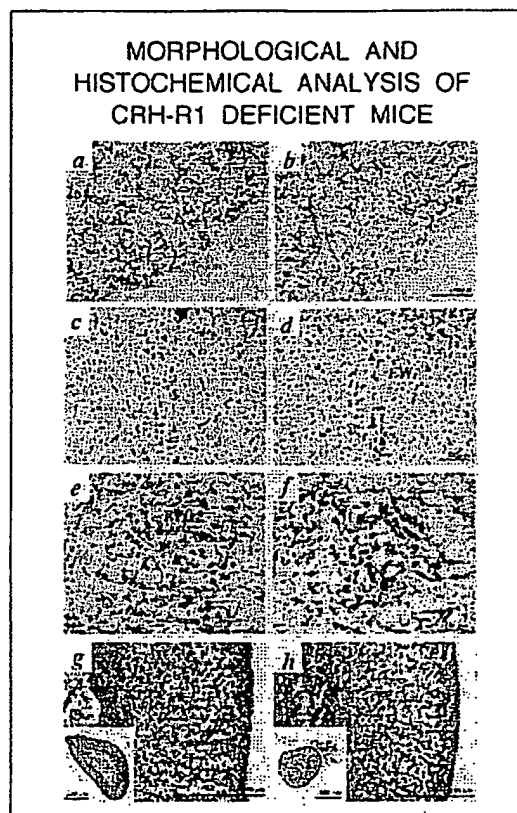


Fig. 20. Morphological and histochemical analysis of CRH receptor-deficient mice: immunohistochemical localization revealed no apparent differences in distribution of corticotrophic cells within the pituitary gland (a = wild-type; b = CRH receptor-deficient mutants). Immunohistochemical localization of urocortin (c = wild-types; d = mutants) is increased in the Edinger-Westphal (EW) nucleus of the mutants. CRH-binding protein is increased in the rostral periolivary (RPO) region. Morphological and histological analysis of the adrenal gland revealed no apparent differences in the size of the subzones of the cortex (r = zona reticularis; f = zona fasciculata; g = zona glomerulosa). Note the marked reduction in the size of the adrenal medulla of mutant mice (adapted from: Timpl et al., 1998 and A. Kresse and M. Müller, unpublished results).

acetylcholine release (Day et al., 1998), it is noteworthy that no significant differences in CRH/CRH-BP or free CRH levels in these brain areas were found between Alzheimer patients and controls.

In the context of investigating increased CRH secretion as a factor in the pathogenesis of depression, drug interventions that lead to increases in CRH-BP would be a worthwhile strategy. Behan et al. (1995b) have pointed out that CRH-BP might exist in various splice forms and that its expression depends on the cell type in the brain. When primary astrocytes are stimulated with forskolin, which directly activates adenyl cyclase to enhance phos-

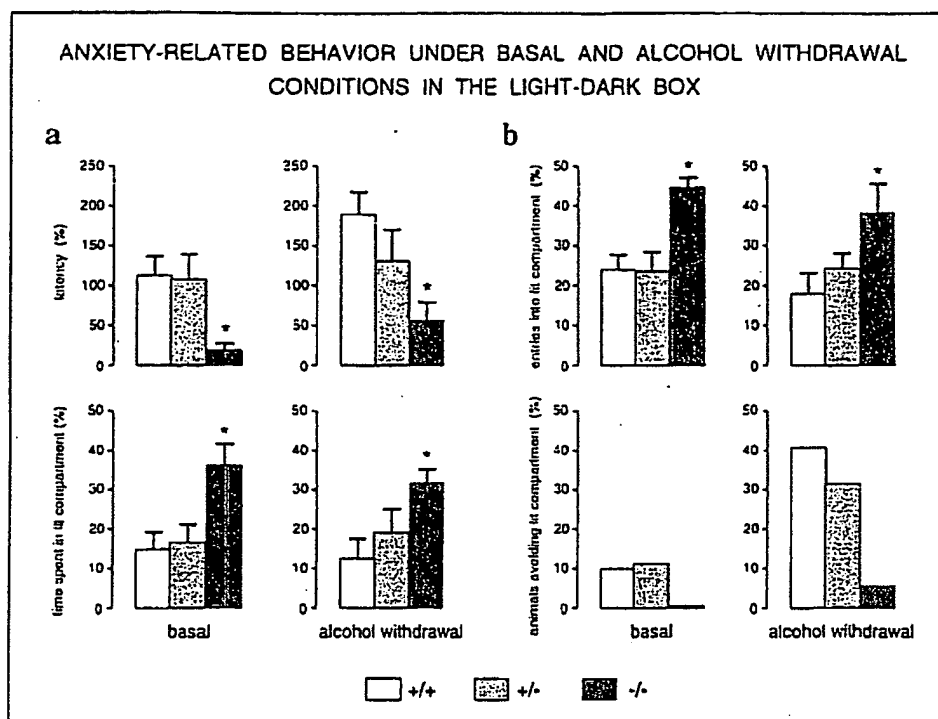


Fig. 21. The time (latency) until the lit compartment was entered is decreased and the time spent in the lit compartment is increased in CRH₁ receptor-deficient mutants (a). After all mice had been subjected to a forced alcohol-drinking procedure and subsequent withdrawal from alcohol, the anxiety-related behavior (latency and time spent in lit compartment) reflected a gene/dosage effect with the intensity of anxiety increasing with increasing CRH₁ receptor levels (wild-type > heterozygous > homozygous). Similar effects were observed when anxiety-related behavior was assessed by scoring the entries into the lit compartment (b, upper panel) or by counting the animals avoiding the lit compartment (b, lower panel) (adapted from: Timpi et al., 1998).

phorylation of CREB via cAMP and PKA, an elevation of CRH-BP mRNA occurs. The same treatment of neuron and astrocyte cultures elicits CRH-BP levels in the media. This finding would suggest that CRH-BP is regulated by a signaling pathway that is also activated through antidepressants. It is tempting to postulate such a mechanism, but it is important to know that the CRH-BP gene does not contain a CRE, and thus enhanced CRH-BP gene expression through phospho-CREB binding to CRE is unlikely. However, an API site has been identified in the CRH-BP promoter sequence, which may have regulatory function. The API site binds API-related proteins (e.g., *jun*, *fos*), and genes regulated by API have been reported to be negatively modulated by ligand-activated GRs (Schüle et al., 1990). Given the high concentration of CRH-BP in areas where GR levels are also high, it is likely that CRH-BP transactivation through API is repressed by formation of an API-GR complex, preventing API-DNA binding and transactivation of API-regulated genes. If such a mechanism exists in the human brain, elevated corticosteroid levels would prevent complexing abundant CRH through suppressing

API-activated CRH-BP expression and consequently enhancing the behavioral and neuroendocrine effects of CRH. Whether such a mechanism is involved is still uncertain, but the question can be addressed by future research.

11. Psychiatric indications for CRH receptor antagonists

The reason for HPA hyperactivity and, in particular, for enhanced production and release of CRH in depression is not yet known. Genetic and experience-related factors may interact to induce manifold changes in corticosteroid receptor signaling. According to the concept developed by Ronald de Kloet et al. (1998), once the balance of MR- and GR-mediated events is disturbed, an individual loses the ability to maintain homeostasis if challenged, for example, by experiencing an adverse life event. This leads to a condition of disturbed neuroendocrine regulation and impaired behavioral adaptation, which, if a certain threshold is surpassed, may

trigger the onset of a psychiatric disorder to which the individual is prone.

The complex regulation of the HPA system provides multiple levels for intervention. A straightforward way to suppress hyperactivity is to use steroid synthesis inhibitors (Murphy, 1991). This strategy has not yet yielded convincing results. Another approach is to use corticosteroid receptor antagonists, which should be effective only under conditions in which central corticosteroid abundance has negative effects on neurocircuitries involved in the development of psychosis. The enhancement of dopaminergic neurotransmission by elevated cortisol secretion is perhaps responsible for delusions in patients with psychotic depression, whereas other symptoms such as psychomotor changes, loss of appetite, and libido and sleep disturbances are more directly caused by CRH. Consequently, a rapid blockade of central cortisol function might be desirable as a first step. Corticosteroid receptor blockers would be a possibility, but as Reul et al. (1993, 1994b) have shown, functional GRs and MRs are essential for conferring long-term antidepressant efficacy, including attenuation of the HPA response to stress. Therefore, only brief treatment with a GR antagonist seems appropriate. Alternatively, a low dosage of dexamethasone, which lowers corticosteroid receptor occupation by suppressing ACTH and cortisol, would be worth clinical testing in patients with psychotic depression. Due to the decreased ability of low dosages of dexamethasone to enter the brain, the loss of cortisol, the main endogenous ligand, is not compensated in the CNS, resulting in a functional antagonism of central GRs and MRs, which increase in number in response to this condition. The good antidepressant efficacy of trimipramine, especially in patients with psychotic depression, is consistent with the reported effects of cortisol on dopaminergic neurotransmission. Trimipramine suppresses the HPA system most effectively in patients with depression (Holsboer-Trachsler et al., 1994; Sonntag et al., 1996).

In general, antidepressants suppress the HPA system after long-term administration, but this effect takes considerable time and is closely linked to the time when the clinical effects of antidepressant drug treatment become apparent. A promising strategy to shorten the time span until antidepressants act by suppressing CRH gene activation and release is the blockade of CRH receptors. All relevant preclinical experiments show that decreasing CRH₁ receptor function either by blockade or by suppressing its synthesis through CRH₁ receptor gene deletion results in a decrease in anxiety in stressed animals. If the preclinical data available for CRH₁ receptor antagonists and those for benzodiazepines are compared, it is not difficult to predict that CRH₁ receptor antagonists will act as anxiolytics. The major difference between these compounds and benzodiazepines is that the latter have some anxiolytic, sedating or hypnotic effects

under any conditions, whereas CRH₁ receptor antagonists are effective only if CRH is hypersecreted. The CRH₁ receptor knockout mice illustrate this, as they still have normal ACTH levels when unstressed, indicating that blockade of this receptor would not interfere with baseline HPA activity. However, these animals show elevated CRH levels in the central amygdala and hypothalamus, raising the question of whether there might be a rebound after cessation of long-term CRH₁ receptor blockade and whether a combination of CRH₁ receptor antagonists and antidepressants would present an advantageous strategy.

Many clinical conditions are accompanied by an exaggerated stress response. Theoretically, all such conditions are potential indications for the use of CRH receptor antagonists. In addition to anxiety and depression, alcohol withdrawal is another very likely indication. This condition has been shown to be associated with excessive HPA activity in both humans and animals. If rats are treated with CRH antagonists the signs of withdrawal are much less severe. Similarly, mouse mutants lacking functional CRH₁ receptors show less severe signs and symptoms during withdrawal from long-term alcohol exposure than do wild-type mice (Timpl et al., 1998). In general, addictive behavior is reinstated by stress in animals and humans after prolonged drug-free periods. A testable hypothesis is the use of CRH₁ receptor antagonists as a preventive strategy to prolong the period of abstinence. Another possible indication for the use of CRH₁ receptor antagonists is a stress-related sleep disturbance, which is typical in depression and under stress, and which can be induced by administration of CRH in rats and humans (Ehlers et al., 1986; Holsboer et al., 1988). The possibility that CRH₁ receptor antagonists may also be of value in treating the neurological consequences of traumatic brain injury has been raised by the group of Nancy Rothwell in Manchester, U.K. After unilateral permanent occlusion of the middle cerebral artery in rats it was observed that the neuronal damage following the ischemic insult was reduced by α -hCRH₁₋₄₁ when given before and after the trauma (Roe et al., 1998). Similarly, in an earlier study, it was possible to reduce the NMDA receptor agonist-induced excitotoxic neuronal damage by α -hCRH₁₋₄₁ administration (Strijbos et al., 1994). These and subsequent experiments indicate that CRH is involved in the neuronal damage that develops after brain injuries, resulting from trauma or excitotoxic causes. These neuronal alterations can be limited by CRH antagonists (Roe et al., 1998). Translated into clinical conditions, these findings suggest that immediate administration of a CRH receptor antagonist, for example after an ischemic insult, might reduce the volume of damaged brain tissue.

Combining molecular genetics with behavioral pharmacology allowed to identify the CRH₁ receptor as a most prominent target for new drugs. Combinatorial

chemistry and high throughput screens led to the generation of a number of candidate compounds that, after structural modification, fulfil the requirements of a CRH₁ receptor antagonist with a promising pharmacological profile. Some of these compounds that emerged from a straightforward 'from bed-to-bench-and-back' strategy are now under clinical investigation.

References

- Aguilera G. Regulation of pituitary ACTH secretion during chronic stress. *Front Neuroendocrinol* 1994;15:321-50.
- Aldenhoff JB, Gruol DL, Rivier J, Vale W, Siggins GR. Corticotrophin-releasing factor decreases postburst hyperpolarizations and excites hippocampal neurons. *Science* 1983;221:875-7.
- Antoni F. Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of 41-residue CRF. *Endocrine Rev* 1986;7:351-8.
- Arato M, Banki CM, Bissette G, Nemeroff CB. Elevated CSF CRH in suicide victims. *Biol Psychiatry* 1989;25:355-9.
- Baldwin HA, Rassnick S, Koob GF, Britton KT. CRF antagonist reverses the 'anxiogenic' response to ethanol withdrawal in the rat. *Psychopharmacology* 1991;103:227-32.
- Banki CM, Bissette G, Arato M, O'Connor L, Nemeroff CB. Cerebrospinal fluid corticotropin-releasing factor-like immunoreactivity in depression and schizophrenia. *Am J Psychiatry* 1987;144:873-7.
- Baram TZ, Hirsch E, Sneed OC 3rd, Schultz L. Corticotropin-releasing hormone-induced seizures in infant rats originate in the amygdala. *Ann Neurol* 1992;31:488-94.
- Baram TZ, Mitchell WG, Haden E. Inhibition of pituitary-adrenal secretion by a corticotropin releasing hormone antagonist in humans. *Mol Psychiatry* 1996a;1:320-4.
- Baram TZ, Koutsoukos Y, Schultz L, Rivier J. The effect of 'Astressin', a novel antagonist of corticotropin releasing hormone (CRH), on CRH-induced seizures in the infant rat: comparison with two other antagonists. *Mol Psychiatry* 1996b;1:223-6.
- Baram TZ, Chalmers DT, Chen C, Koutsoukos Y, de Souza EB. The CRF₁ receptor mediates the excitatory actions of corticotropin releasing factor (CRF) in the developing rat brain: in vivo evidence using a novel, selective, non-peptide CRF receptor antagonist. *Brain Res* 1997;770:89-95.
- Birder N, Stec SM, Montkowski A, Holshofer F, Reul JMHM. Endocrine profile and neuroendocrine challenge test in transgenic mice expressing antisense RNA against the glucocorticoid receptor. *Neuroendocrinology* 1997;66:212-20.
- Behan DP, Heinrichs SC, Troncoso JC, Liu X-J, Kowas CH, Ling N, de Souza EB. Displacement of corticotropin releasing factor from its binding protein as a possible treatment for Alzheimer's disease. *Nature* 1995a;378:284-7.
- Behan DP, Maciejewski D, Chalmers D, de Souza EB. Corticotropin releasing factor binding protein (CRF-BP) is expressed in neuronal and astrocytic cells. *Brain Res* 1995b;698:259-64.
- Behan DP, Khongsuly O, Owens MJ, Chung HD, Nemeroff CB, de Souza EB. Corticotropin-releasing factor (CRF), CRF-binding protein (CRF-BP), and CRF/CRF-BP complex in Alzheimer's disease and control postmortem human brain. *J Neurochem* 1997;68:2053-60.
- Behl C. Alzheimer's disease and oxidative stress: implications for novel therapeutic approaches. *Prog Neurobiol* 1998;56:1-23.
- Bissette G, Reynolds GP, Kilts CD, Widerlov W, Nemeroff CB. Corticotropin-releasing factor-like immunoreactivity in senile dementia of the Alzheimer type. *JAMA* 1985;254:3067-9.
- Bliss TVP, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 1993;361:31-9.
- Bourgeois S, Gruol DJ, Newby RF, Rajah FM. Expression of an mdr gene is associated with a new form of resistance to dexamethasone-induced apoptosis. *Mol Endocrinol* 1993;7:840-51.
- Bradbury M, Akana SF, Dallman MF. Roles of type I and type 2 corticosteroid receptors in the regulation of basal activity in the hypothalamic-pituitary-adrenal axis during the diurnal trough and peak: evidence for a non-additive effect of combined receptor occupation. *Endocrinology* 1994;134:1286-96.
- Britton DR, Koob GF, River H, Vale W. Intraventricular corticotropin-releasing factor enhances behavioral effects of novelty. *Life Sci* 1982;31:363-7.
- Britton KT, Lee G, Vale W, Rivier J, Koob GF. Corticotropin-releasing factor (CRF) receptor antagonist blocks activating and 'anxiogenic' actions of CRF in the rat. *Brain Res* 1986;369:303-6.
- Butler PD, Weiss JM, Stout JC, Nemeroff CB. Corticotropin-releasing factor produces fear-enhancing and behavioral activating effects following infusion into the locus coeruleus. *J Neurosci* 1990;10:176-83.
- Cai Z, McCaslin PP. Amitriptyline, desipramine, cyproheptadine and carbamazepine, in concentrations used therapeutically, reduce kainate- and *N*-methyl-D-aspartate-induced intracellular Ca^{2+} levels in neuronal culture. *Eur J Pharmacol* 1992;219:53-7.
- Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, Meaney MJ. Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proc Natl Acad Sci USA* 1998;95:5335-40.
- Chalmers DT, Lovenberg TW, De Souza EB. Localization of novel corticotropin-releasing factor receptor (CRF₂) mRNA expression to specific subcortical nuclei in rat brain: Comparison with CRF₁ receptor mRNA expression. *J Neurosci* 1995;15:6340-50.
- Chalmers DT, Lovenberg TW, Grigoriadis DE, Behan DP, De Souza EB. Corticotropin-releasing factor receptors: from molecular biology to drug design. *Trends Pharmacol Sci* 1996;17:166-72.
- Chang CP, Pearce RV 2nd, O'Connell S, Rosenfeld MG. Identification of a seven transmembrane helix receptor for corticotropin-releasing factor and sauvagine in mammalian brain. *Neuron* 1993;11:1187-95.
- Charlton BG, Cheetham SC, Horton RW, Katona CLE, Crompton MR, Ferrier IN. Corticotropin-releasing factor immunoreactivity in post-mortem brain from depressed suicides. *J Psychopharmacol* 1988;2:13-8.
- Chen C, Dagnino R, De Souza EB, Grigoriadis DE, Huang CQ, Kim K-L, Liu T, Moran T, Webb TR, Witten JP, Xie YF, McCarthy JR. Design and synthesis of a series of non-peptide high affinity human corticotropin releasing factor-1 receptor antagonists. *J Med Chem* 1996;39:4358-60.
- Chen R, Lewis KA, Perrin MH, Vale WW. Expression cloning of a human corticotropin-releasing factor receptor. *Proc Natl Acad Sci USA* 1993;90:8967-71.
- Choi JJ, Huang G-J, Shafik E, Wu W-H, McArdle JJ. Imipramine's selective suppression of an L-type calcium channel in neurons of murine dorsal root ganglia involves G proteins. *J Pharmacol Exp Ther* 1992;263:49-53.
- Collins S, Caron MG, Lefkowitz RJ. β_2 -adrenergic receptors in hamster smooth muscle cells are transcriptionally regulated by glucocorticoids. *J Biol Chem* 1988;263:9067-70.
- Coplan JD, Andrews MW, Rosenblum LA, Owens MJ, Friedman S, Gorman JM, Nemeroff CB. Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult non-human primates exposed to early-life stressors: implications for the pathophysiology of mood and anxiety disorders. *Proc Natl Acad Sci USA* 1996;93:1619-23.
- Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR, Bertino JR. Multidrug-resistance gene (P-gly-

- coprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc Natl Acad Sci USA* 1989;86:695-8.
- Costu A, Yassin SA, Hucks D, Forsling ML, Besser GM, Grossman A. Differential effects of neuroexcitatory amino acids on corticotropin-releasing hormone-41 and vasopressin release from rat hypothalamic explants. *Endocrinology* 1992;131:2595-602.
- Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. *Science* 1993;262:689-95.
- Dallman MF. Stress Update. Adaptation of the hypothalamic-pituitary-adrenal axis to chronic stress. *Trends Endocrinol Metab* 1993;4:62-9.
- Dautzenberg FM, Dietrich K, Palchoudhuri MR, Spiess J. Identification of two corticotropin-releasing factor receptors from *Xenopus laevis* with high ligand selectivity: unusual pharmacology of the type 1 receptor. *J Neurochem* 1997;69:1640-9.
- Dautzenberg FM, Wille S, Lohmann R, Spiess J. Mapping of the ligand-selective domain of the *Xenopus laevis* corticotropin-releasing factor receptor 1: Implications for the ligand-binding site. *Proc Natl Acad Sci USA* 1998;95:4941-6.
- Day JC, Koehl M, Deroche V, Le Moal M, Stefania M. Prenatal stress enhances stress- and corticotropin-releasing factor-induced stimulation of hippocampal acetylcholine release in adult rats. *J Neurosci* 1998;18:1886-92.
- De Bellis MD, Gold PW, Geraciotti TD Jr, Listwak SJ, Kling MA. Association of fluoxetine treatment with reductions in CSF concentrations of corticotropin-releasing hormone and arginine vasopressin in patients with major depression. *Am J Psychiatry* 1993;150:656-7.
- De Goeij DC, Kvaternik R, Whitnall MH, Jezova D, Berkenbosch F, Tilders FJ. Repeated stress-induced activation of corticotropin-releasing factor neurons enhances vasopressin stores and colocalization with corticotropin-releasing factor in the median eminence of rats. *Neuroendocrinology* 1991;53:150-9.
- De Kloet ER, Vreugdenhil E, Oitzl MS, Joels M. Brain corticosteroid receptor balance in health and disease. *Endocrine Rev* 1998;19:269-301.
- De Souza EB, Whitehouse PJ, Kuhar MJ, Price DL, Vale WW. Reciprocal changes in corticotropin-releasing factor (CRF)-like immunoreactivity and CRF receptors in cerebral cortex of Alzheimer's disease. *Nature* 1986;319:593-5.
- Dijkstra I, Tilders FJH, Aguilera G, Kiss A, Rubaud-Duché C, Barden N, Karanth S, Holsboer F, Reul JMHM. Reduced activity of hypothalamic corticotropin-releasing hormone neurons in transgenic mice with impaired glucocorticoid receptor function. *J Neurosci* 1998;18:3009-918.
- Drouin J, Maira M, Philips A. Novel mechanism of action for Nur77 and antagonist by glucocorticoids: a convergent mechanism for CRH activation and glucocorticoid repression of POMC gene transcription. *J Steroid Biochem Mol Biol* 1998;65:59-63.
- Duman RS, Nestler EJ. Signal transduction pathways for catecholamine receptors. In: Bloom FE, Kupfer DJ, editors. *Psychopharmacology: The Fourth Generation of Progress*. New York, Raven Press, 1995:303-20.
- Duman RS, Heninger GR, Nestler EJ. A molecular and cellular theory of depression. *Arch Gen Psychiatry* 1997;54:597-606.
- Dunn AJ, File SE. Corticotropin-releasing factor has an anxiogenic action in the social interaction test. *Hormones Behav* 1987;21:193-202.
- Dunn AJ, Berridge CW. Physiological and behavioral response to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? *Brain Res Rev* 1990;15:71-100.
- Ehlers CL, Reed TK, Henriksen SJ. Effects of corticotropin-releasing factor and growth hormone-releasing factor on sleep and activity in rats. *Neuroendocrinology* 1986;42:467-74.
- Gallagher M, Chiba AA. The amygdala and emotion. *Curr Opin Neurobiol* 1996;6:221-7.
- Geraciotti TD Jr, Orth DN, Ekhtori NN, Blumenkopf B, Loosen PT. Serial cerebrospinal fluid corticotropin-releasing hormone concentrations in healthy and depressed humans. *J Clin Endocrinol Metab* 1992;74:1325-30.
- Geraciotti TD Jr, Loosen PT, Orth DN. Low cerebrospinal fluid corticotropin-releasing hormone concentrations in eucortisolemic depression. *Biol Psychiatry* 1997;42:166-74.
- Gold PW, Chrousos G, Kellner C, Post R, Roy A, Augerinos P, Schulte H, Oldfield E, Loriaux DL. Psychiatric implications of basic and clinical studies with corticotropin-releasing factor. *Am J Psychiatry* 1984;141:619-27.
- Griebel G, Perrault G, Sanger DJ. Characterization of the behavioral profile of the non-peptide CRF receptor antagonist CP-154,526 in anxiety models in rodents. Comparison with diazepam and buspirone. *Psychopharmacology* 1998;138:55-66.
- Hauger RL, Aguilera G. Regulation of pituitary corticotropin-releasing hormone receptors by CRH: interaction with vasopressin. *Endocrinology* 1993;133:1708-14.
- Hauger RL, Irwin MR, Lorang M, Aguilera G, Brown MR. High intracerebral levels of CRH result in CRH receptor downregulation in the amygdala and neuroimmune desensitization. *Brain Res* 1993;616:283-92.
- Hauger RL, Dautzenberg FM, Flaccus A, Liepold T, Spiess J. Regulation of corticotropin-releasing factor receptor function in human Y-79 retinoblastoma cells: rapid and reversible homologous desensitization but prolonged recovery. *J Neurochem* 1997;68:2308-16.
- Heck S, Kullmann M, Gast A, Pouta H, Rahmsdorf HJ, Herrlich P, Cato AC. A distinct modulating domain in glucocorticoid receptor monomers in the repression of activity of the transcription factor AP-1. *EMBO J* 1994;13:4087-95.
- Heinrichs SC, Merlo-Pich E, Miczek KA, Britton KT, Koob GF. Corticotropin-releasing factor antagonist reduces emotionality in socially defeated rats via direct neurotropic action. *Brain Res* 1992;581:190-7.
- Heinrichs SC, Menzaghi F, Merlo-Pich E, Baldwin HA, Rassnick S, Britton KT, Koob GF. Anti-stress action of a corticotropin-releasing factor antagonist on behavioral reactivity to stressors of varying type and intensity. *Neuropsychopharmacology* 1994;11:179-86.
- Heinrichs SC, Stenzel-Poore MP, Gold LH, Battenberg E, Bloom FE, Koob GF, Vale WW, Merlo-Pich E. Learning impairment in transgenic mice with central overexpression of corticotropin-releasing factor. *Neuroscience* 1996;74:303-11.
- Heinrichs SC, Lapansky J, Lovenberg TW, De Souza EB, Chalmers DT. Corticotropin-releasing factor CRF₁, but not CRF₂, receptors mediate anxiogenic-like behavior. *Regul Peptides* 1997;71:15-21.
- Heuser I, Yassouridis A, Holsboer F. The combined dexamethasone/CRH test: A refined laboratory test for psychiatric disorders. *J Psychiatric Res* 1994;28:341-56.
- Heuser IJE, Schweiger U, Gotthardt U, Schmitter J, Lammers CH, Dertling M, Yassouridis A, Holsboer F. Pituitary-adrenal-system regulation and psychopathology during amitriptyline treatment in elderly depressed patients and in normal comparison subjects. *Am J Psychiatry* 1996;153:93-9.
- Heuser I, Deuschle M, Weber B, Stalla GK, Holsboer F. Increased activity of the hypothalamus-pituitary-adrenal system after treatment with the mineralocorticoid receptor antagonist spironolactone. *Psychoneuroendocrinology* 1998; in press.
- Holsboer F. Prediction of clinical course by dexamethasone suppression test (DST) response in depressed patients—physiological and clinical construct validity of the DST. *Pharmacopsychiatry* 1983;16:186-91.
- Holsboer F. Neuroendocrinology of mood disorders. In: Bloom FE, Kupfer DJ, editors. *Psychopharmacology: The Fourth Generation of Progress*. New York, Raven Press, 1995a:957-69.
- Holsboer F, Barden N. Antidepressants and HPA regulation. *Endocrine Rev* 1996;17:187-205.
- Holsboer F, von Bardeleben U, Gerken A, Stalla GK, Müller OA. Blunted corticotropin and normal cortisol response to human corti-

- corticotropin-releasing factor in depression. *N Engl J Med* 1984a;311:1127.
- Holsboer F, Müller OA, Doerr HG, Sippell WG, Stalla GK, Gerken A, Steiger A, Boll E, Benkert O. ACTH and multiteroid responses to corticotropin-releasing factor in depressive illness: relationship to multiteroid responses after ACTH stimulation and dexamethasone suppression. *Psychoneuroendocrinology* 1984b;9:147-69.
- Holsboer F, Gerken A, von Bardeleben U, Grimm W, Beyer H, Müller OA, Stalla GK. Human corticotropin-releasing hormone in depression. *Biol Psychiatry* 1986;21:601-11.
- Holsboer F, von Bardeleben U, Buller R, Heuser I, Steiger A. Stimulation response to corticotropin-releasing hormone (CRH) in patients with depression, alcoholism and panic disorder. *Hormone Metab Res* 1987a;19:80-8.
- Holsboer F, von Bardeleben U, Wiedemann K, Müller OA, Stalla GK. Serial assessment of corticotropin-releasing hormone response after dexamethasone in depression - Implications for pathophysiology of DST nonsuppression. *Biol Psychiatry* 1987b;22:228-34.
- Holsboer F, von Bardeleben U, Steiger A. Effects of intravenous corticotropin-releasing hormone upon sleep-related growth hormone surge and sleep EEG in man. *Neuroendocrinology* 1988;48:32-8.
- Holsboer F, Spengler D, Heuser I. The role of corticotropin-releasing hormone in the pathogenesis of Cushing's disease, anorexia nervosa, alcoholism, affective disorders and dementia. *Prog Brain Res* 1992;93:385-417.
- Holsboer F, Lauer CJ, Schreiber W, Krieg J-C. Altered hypothalamic-pituitary-adrenocortical regulation in healthy subjects at high familial risk for affective disorders. *Neuroendocrinology* 1995b;62:340-7.
- Holsboer-Trachsler E, Hemminger U, Hatzinger M, Seifritz E, Gerhard U, Hobi V. Sleep deprivation and bright light as potential augmenters of antidepressant drug treatment—neurobiological and psychometric assessment of course. *J Psychiatric Res* 1994;28:381-99.
- Hsu DT, Chen F-L, Takahashi LK, Kalin NH. Rapid stress-induced elevations in corticotropin-releasing hormone mRNA in rat central amygdala nucleus and hypothalamic paraventricular nucleus: An in situ hybridization analysis. *Brain Res* 1998;788:305-10.
- Hucks D, Lowther S, Crompton MR, Kutona CLE, Horton RW. Corticotropin-releasing factor binding sites in cortex of depressed suicides. *Psychopharmacology* 1997;134:174-8.
- Imaki T, Vale W. Chlordiazepoxide attenuates stress-induced accumulation of corticotropin-releasing factor mRNA in the paraventricular nucleus. *Brain Res* 1993;623:223-8.
- Iredale PA, Terwilliger R, Widnell KL, Nestler EJ, Duman RS. Differential regulation of corticotropin-releasing factor₁ receptor expression by stress and agonist treatments in brain and cultured cells. *Mol Pharmacol* 1996;50:1103-10.
- Iredale PA, Bunday R, Duman RS. Phorbol ester and calcium regulation of corticotropin-releasing factor receptor 1 expression in a neuronal cell line. *J Neurochem* 1997;69:1912-19.
- Jezova D, Oprsalova Z. Adrenocorticotropin release induced by *N*-methyl-D-aspartate or stress: mediation by the arcuate postrema. *J Neuroendocrinol* 1992;4:145-7.
- Joanny P, Steinberg J, Oliver C, Grino M. Glutamate and *N*-methyl-D-aspartate stimulate rat hypothalamic corticotropin-releasing factor secretion in vitro. *J Neuroendocrinol* 1997;9:93-7.
- Joshi J, Sabol SL. Proenkephalin gene expression in C6 rat glioma cells: potentiation of cyclic adenosine 3',5'-monophosphate-dependent transcription by glucocorticoids. *Mol Endocrinol* 1991;5:1069-80.
- Kalin NH. Behavioral and endocrine studies of corticotropin-releasing hormone in primates. In: De Souza EB, Nemeroff CB, editors. *Corticotropin-Releasing Factor: Basic and Clinical Studies of a Neuropeptide*. Boca Raton: CRC Press, 1990:275-89.
- Kendler KS. Major depression and the environment: a psychiatric genetic perspective. *Pharmacopsychiatry* 1998;31:5-9.
- Kendler KS, Karkowski-Shuman L. Stressful life events and genetic liability to major depression: genetic control of exposure to the environment? *Psychol Med* 1997;27:539-47.
- Koob GF, Bloom FE. Corticotropin-releasing factor and behavior. *Fed Proc* 1985;44:259-63.
- Koob GF, Heinrichs SC, Merlo-Pich E, Menzaghi F, Baldwin H, Miczek H, Britton KT. The role of corticotropin-releasing factor in behavioural response to stress. In: De Souza EB, Nemeroff CB, editors. *Corticotropin-Releasing Factor: Basic and Clinical Studies of a Neuropeptide*. Boca Raton: CRC Press, 1993:277-95.
- Koob GF, Heinrichs SC, Menzaghi P, Pich EM, Britton KT. Corticotropin-releasing factor, stress and behavior. *Semin Neurosci* 1994;6:221-9.
- Korte SM, Korte-Bouws GAH, Bohus B, Koob GF. Effect of corticotropin-releasing factor antagonist on behavioral and neuroendocrine responses during exposure to defensive burying paradigm in rats. *Physiol Behav* 1994;56:115-20.
- Kostich WA, Chen A, Sperle K, Largent BL. Molecular identification and analysis of a novel human corticotropin-releasing factor (CRF) receptor: the CRF₂ receptor. *Mol Endocrinol* 1998;12:1077-85.
- Kupfer DJ. Management of recurrent depression. *J Clin Psychiatry* 1993;54 Suppl 2:29-33.
- LaBar KS, Gatenby JC, Gore JC, LeDoux JE, Phelps EA. Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. *Neuron* 1998;20:937-45.
- Ladd CO, Owens MJ, Nemeroff CB. Persistent changes in corticotropin-releasing factor neuronal systems induced by maternal deprivation. *Endocrinology* 1996;137:1212-18.
- Landgraf R, Gerstberger R, Montkowski A, Probst JC, Wotjak CT, Holsboer F, Engelmann M. VI vasopressin receptor antisense oligodeoxynucleotide into septum reduces vasopressin binding, social discrimination abilities and anxiety-related behavior in rats. *J Neurosci* 1995;15:4250-58.
- Lauer CJ, Schreiber W, Modell S, Holsboer F, Krieg J-C. The Munich vulnerability study on affective disorders: overview of the cross-sectional observations in index investigation. *J Psychiatric Res* 1998;32:393-401.
- Leake A, Perry EK, Perry RH, Fairbairn AF, Ferrier IN. Cortical concentrations of corticotropin-releasing hormone and its receptor in Alzheimer type dementia and major depression. *Biol Psychiatry* 1990;28:603-8.
- Lefkowitz RJ. G protein-coupled receptor kinase. *Cell* 1993;74:409-12.
- Liebsch G, Landgraf R, Gerstberger R, Probst JC, Wotjak CT, Engelmann M, Holsboer F, Montkowski A. Chronic infusion of a CRH₁ receptor antisense oligodeoxynucleotide into the central nucleus of the amygdala reduced anxiety-related behavior in socially defeated rats. *Regul Peptides* 1995;59:229-39.
- Liebsch G, Montkowski A, Holsboer F, Landgraf R. Behavioural profiles of two Wistar rat lines selectively bred for high or low anxiety-related behaviour. *Behav Brain Res* 1998;94:301-10.
- Liebsch G, Landgraf R, Engelmann M, Lorsch P, Holsboer F. Differential behavioural effects of chronic infusion of CRH₁ and CRH₂ receptor antisense oligonucleotides into the rat brain. *J Psychiatric Res* 1999;33:153-163.
- Linthorst ACE, Flachskamm C, Hopkins SJ, Hoadley ME, Labeur MS, Holsboer F, Reul JMHM. Long-term intracerebroventricular infusion of corticotropin-releasing hormone alters neuroendocrine, neurochemical, autonomic, behavioral, and cytokine responses to a systemic inflammatory challenge. *J Neurosci* 1997;17:4448-60.
- Lisansky J, Peake GT, Strassman RJ, Qualls C, Meikle AW, Risch SC, Fava GA, Zowin-Brazis M, Hochla P, Britton D. Augmented pituitary corticotropin response to a threshold dosage of human corticotropin-releasing hormone in depressives pre-treated with meprobamate. *Arch Gen Psychiatry* 1989;46:641-9.
- Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky PM, Meaney MJ. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science* 1997;277:1659-62.

- Lovenberg TW, Liaw CW, Grigoridis DE, Clevenger W, Chalmers DT, De Souza EB, Oltersdorf T. Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. *Proc Natl Acad Sci USA* 1995;92:836-40.
- Lundkvist J, Chai Z, Tehraniian R, Hasunvan H, Bartfai T, Jenck F, Widmer U, Moreau JL. A non-peptidic corticotropin releasing factor receptor antagonist attenuates fever and exhibits anxiolytic-like activity. *Eur J Pharmacol* 1996;309:195-200.
- Luo X, Kiss A, Makara G, Lolait SJ, Aguilera G. Stress-specific regulation of corticotropin releasing hormone receptor expression in the paraventricular and supraoptic nuclei of the hypothalamus in the rat. *J Neuroendocrinol* 1994;6:689-96.
- Malkoski SP, Handanos CM, Dorin RI. Localization of a negative glucocorticoid response element of the human corticotropin releasing hormone gene. *Mol Cell Endocrinol* 1997;127:189-99.
- Mansbach RS, Brooks EN, Chen YL. Antidepressant-like effects of CP-154,526, a selective CRF₁ receptor antagonist. *Eur J Pharmacol* 1997;323:21-6.
- Mecker RB, Greenwood RS, Hayward JN. Glutamate receptors in the rat hypothalamus and pituitary. *Endocrinology* 1994;134:621-9.
- Meijer OC, de Lange ECM, Breimer DD, de Boer AG, Workel JO, de Kloet ER. Penetration of dexamethasone into brain glucocorticoid targets is enhanced in mdrlA P-glycoprotein knockout mice. *Endocrinology* 1998;139:1789-93.
- Melia KR, Duman RS. Involvement of corticotropin-releasing factor in chronic stress regulation of the brain noradrenergic system. *Proc Natl Acad Sci U.S.A.* 1991;88:8382-6.
- Menzaghi F, Howard RL, Heinrichs SC, Valer W, Rivier J, Koob GF. Characterization of a novel and potent corticotropin-releasing factor antagonist in rats. *J Pharmacol Exp Ther* 1994;269:564-72.
- Merchenthaler J. Corticotropin-releasing factor (CRF)-like immunoreactivity in the rat central nervous system. Extrahypothalamic distribution. *Peptides* 1994;5:53-69.
- Meyer TE, Habener JF. Cyclic adenosine 3',5'-monophosphate response element binding protein (CREB) and related transcription-activating deoxyribonucleic acid-binding proteins. *Endocrine Rev* 1993;14:269-90.
- Michelson D, Galliven E, Hill L, Demitrack M, Chrousos G, Gold P. Chronic imipramine is associated with diminished hypothalamic-pituitary-adrenal axis responsiveness in healthy humans. *J Clin Endocrinol Metab* 1997;82:2601-6.
- Mobley PL, Sulzer D. Adrenal corticoids regulate sensitivity of nor-adrenaline receptor-coupled adenylate cyclase in brain. *Nature* 1980;286:608-9.
- Modell S, Yassouridis A, Huber J, Holsboer F. Corticosteroid receptor function is decreased in depressed patients. *Neuroendocrinology* 1997;65:216-22.
- Modell S, Lauer CJ, Schreiber W, Huber J, Krieg J-C, Holsboer F. Hormonal response pattern in the combined DEX-CRH test is stable over time in subjects at high familial risk for affective disorders. *Neuropsychopharmacology* 1998;18:253-62.
- Molchan SE, Hill JL, Martinez RA, Lawlor BA, Mellow AM, Rubinow DR, Bissette G, Nemeroff CB, Sunderland T. CSF somatostatin in Alzheimer's disease and major depression: Relationship to hypothalamic-pituitary-adrenal axis and clinical measures. *Psychoneuroendocrinology* 1993;18:509-19.
- Montkowski A, Barden N, Wotjak C, Stoe I, Ganster J, Meaney M, Engelmann M, Reul JMH, Landgraf R, Holsboer F. Long-term antidepressant treatment reduces behavioural deficits in transgenic mice with impaired glucocorticoid receptor function. *J Neuroendocrinol* 1995;7:841-5.
- Moreau JL, Kilpatrick G, Jenck F. Urocortin, a novel neuropeptide with anxiogenic-like properties. *Neuroreport* 1997;8:1697-1701.
- Mori S, Zanardi R, Popoli M, Garbini S, Brunello N, Smeraldi E, Racagni G, Perez J. cAMP-dependent phosphorylation system after short and long-term administration of moclobemide. *J Psychiatric Res* 1998;32:111-5.
- Murphy BEP. Steroids and depression. *J Steroid Biochem Mol Biol* 1991;38:537-59.
- Murphy BEP and Connolly OM. Neuroendocrine regulation of the hypothalamic-pituitary-adrenal axis by the nurr1/nur77 subfamily of nuclear receptors. *Mol Endocrinol* 1997;11:39-47.
- Nemeroff CB, Widerlov E, Bissette G, Walleus H, Karlsson I, Eklund K, Killa DC, Loosen PT, Vale W. Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* 1984;226:1342-4.
- Nemeroff CB, Owens MJ, Bissette G, Andorn AC, Stanley M. Reduced corticotropin releasing factor binding sites in the frontal cortex of suicide victims. *Arch Gen Psychiatry* 1988;45:577-9.
- Nestler EJ, Terwilliger RZ, Duman RS. Chronic antidepressant administration alters the subcellular distribution of cyclic AMP-dependent protein kinase in rat frontal cortex. *J Neurochem* 1989;53:1644-7.
- Nibuya M, Nestler EJ, Duman RS. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *J Neurosci* 1996;16:2365-72.
- Orth DN. Corticotropin-releasing hormone in humans. *Endocrine Rev* 1992;13:164-91.
- Owens MJ, Nemeroff CB. The physiology and pharmacology of corticotropin-releasing factor. *Pharmacol Rev* 1992;43:423-73.
- Owens MJ, Bissette G, Nemeroff CB. Acute effects of alprazolam and adiazepam on the concentration of corticotropin-releasing factor in the rat brain. *Synapse* 1989;4:196-202.
- Parkes D, Rivest S, Lee S, Rivier C, Vale W. Corticotropin-releasing factor activates c-fos, NGF-B, and corticotropin-releasing factor gene expression within the paraventricular nucleus of the rat hypothalamus. *Mol Endocrinol* 1993;7:1357-67.
- Patchev VK, Shoaib M, Holsboer F, Almeida OFX. The neurosteroid tetrahydroprogesterone counteracts CRH-induced anxiety and alters the release and gene expression of CRH in the rat hypothalamus. *Neuroscience* 1994a;62:265-71.
- Patchev VK, Karalis K, Chrousos GP. Effects of excitatory amino acid transmitters on hypothalamic corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) release in vitro: implications in pituitary-adrenal regulation. *Brain Res* 1994b;633:312-6.
- Patchev VK, Montkowski A, Rouskova D, Koranyi L, Holsboer F, Almeida OFX. Neonatal treatment of rats with the neurosteroid tetrahydrodeoxycorticosterone (THDOC) abolishes the behavioral and neuroendocrine consequences of adverse early life events. *J Clin Invest* 1997;99:962-6.
- Paul SM, Purdy RH. Neuroactive steroids. *FASEB J* 1992;6:2311-22.
- Pepin MC, Beaulieu S, Barden N. Antidepressants regulate glucocorticoid receptor messenger RNA concentrations in primary neuronal cultures. *Mol Brain Res* 1989;6:77-83.
- Pepin MC, Pothier F, Burden N. Impaired type II glucocorticoid-receptor function in mice bearing antisense RNA transgene. *Nature* 1992;355:725-8.
- Perez J, Tinelli D, Brunello N, Racagni G. cAMP-dependent phosphorylation of soluble and crude microtubule fractions of rat cerebral cortex after prolonged desmethylimipramine treatment. *Eur J Pharmacol* 1989;172:305-16.
- Perez J, Tinelli D, Bianchi E, Brunello N, Racagni G. cAMP binding proteins in the rat cerebral cortex after administration of selective 5-HT and NE reuptake blockers with antidepressant activity. *Neuropsychopharmacology* 1991;4:57-64.
- Perrin MH, Donaldson CJ, Chen R, Lewis KA, Vale WW. Cloning and functional expression of a rat brain corticotropin releasing factor (CRF) receptor. *Endocrinology* 1993;133:3058-61.
- Perrin M, Donaldson C, Chen R, Blount A, Berggren T, Bilezikian L, Sawchenko P, Vale W. Identification of a second corticotropin-releasing factor receptor gene and characterization of a cDNA expressed in heart. *Proc Natl Acad Sci USA* 1995;92:2969-73.
- Phi Van L, Spengler D, Holsboer F. Glucocorticoid repression of cAMP-dependent hCRH gene promoter activity in a transfected mouse anterior pituitary cell line. *Endocrinology* 1990;127:1412-8.

- Pitts AF, Samuelson SD, Meller WH, Bissette G, Nemeroff CB, Kathos RG. Cerebrospinal fluid corticotropin-releasing hormone, vasopressin, and oxytocin concentrations in treated patients with major depression and controls. *Biol Psychiatry* 1995;38:330-5.
- Plotsky PM. Noli disturbare circulos meos: Integrative role for CRF in organization of the stress response. In: Nappi G, et al. editors. *Stress and the Aging Brain*. New York: Raven Press, 1990.
- Pomara N, Singh RR, Deptula D, Lewitt PA, Bissette G, Stanley M, Nemeroff CB. CSF corticotropin-releasing factor (CRF) in Alzheimer's disease: its relationship to severity of dementia and monoamine metabolites. *Biol Psychiatry* 1989;26:500-4.
- Potter E, Behan DP, Fischer WH, Linton EA, Lowry PJ, Vale WW. Cloning and characterization of the cDNAs for human and rat corticotropin releasing factor-binding proteins. *Nature* 1991;349:423-6.
- Potter E, Behan DP, Linton EA, Lowry PJ, Sawchenko PE, Vale WW. The central distribution of a corticotropin-releasing factor (CRF)-binding protein predicts multiple sites and modes of interaction with CRF. *Proc Natl Acad Sci USA* 1992;89:4192-6.
- Potter E, Sutton S, Donaldson C, Chen R, Perrin M, Lewis K, Sawchenko PE, Vale W. Distribution of corticotropin-releasing factor receptor mRNA expression in the rat brain and pituitary. *Proc Natl Acad Sci USA* 1994;91:8777-81.
- Price ML, Curtis AL, Kirby LG, Valentino RJ, Lucki I. Effects of corticotropin-releasing factor on brain serotonergic activity. *Neuropsychopharmacology* 1998;18:492-502.
- Purba JS, Hoogendijk WJG, Hofman MA, Swaab DF. Increased number of vasopressin- and oxytocin-expressing neurons in the paraventricular nucleus of the hypothalamus in depression. *Arch Gen Psychiatry* 1996;53:137-43.
- Raadshere FC, Hoogendijk WJG, Stum FC, Tilders FJH, Swaab DF. Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. *Neuroendocrinology* 1994;60:433-6.
- Rabadan-Diehl C, Makara G, Kiss A, Zelena D, Aguilera G. Regulation of pituitary corticotropin-releasing hormone (CRH) receptor mRNA and CRH binding during adrenalectomy: role of glucocorticoids and hypothalamic factors. *J Neuroendocrinol* 1997;9:689-97.
- Racagni G, Brunello N, Tinelli D, Perez J. New biochemical hypotheses on the mechanism of action of antidepressant drugs: cAMP-dependent phosphorylation system. *Pharmacopsychiatry* 1992;25:51-5.
- Rainnie DG, Fernhout BJ, Shinnick-Gallagher P. Differential actions of corticotropin-releasing factor on basolateral and central amygdaloid neurons in vitro. *J Pharmacol Exp Ther* 1992;263:846-58.
- Rangarajan PN, Umesono K, Evans RM. Modulation of glucocorticoid receptor function by protein kinase A. *Mol Endocrinol* 1992;6:1451-7.
- Rassnick S, Heinrichs SC, Britton KT, Koob GF. Microinjection of a corticotropin-releasing factor antagonist into the central nucleus of the amygdala reverses anxiogenic-like effects of ethanol withdrawal. *Brain Res* 1993;605:25-32.
- Reichardt HM, Kaestner KH, Tuckermann J, Kretz O, Wessely O, Bock R, Gass P, Schmid W, Herrlich P, Angel P, Schütz G. DNA binding of the glucocorticoid receptor is not essential for survival. *Cell* 1998;93:531-41.
- Reul JMHM, Stec I, Söder M, Holsboer F. Chronic treatment of rats with the antidepressant amitriptyline attenuates the activity of the hypothalamic-pituitary-adrenocortical system. *Endocrinology* 1993;133:312-20.
- Reul JMHM, Stec I, Wiegers GJ, Labeur MS, Linthorst ACE, Arzt E, Holsboer F. Prenatal immune challenge alters the hypothalamic-pituitary-adrenocortical axis in adult rats. *J Clin Invest* 1994a;93:2600-7.
- Reul JMHM, Labeur MS, Grigoriadis DE, De Souza EB, Holsboer F. Hypothalamic-pituitary-adrenocortical axis changes in the rat after long-term treatment with the reversible monoamine oxidase-A inhibitor moclobemide. *Neuroendocrinology* 1994b;60:509-19.
- Reyes A, Luckhaus J, Ferin M. Unexpected inhibitory action of N-methyl-D,L-aspartate on luteinizing hormone release in adult ovariectomized rhesus monkeys: a role of the hypothalamic-adrenal axis. *Endocrinology* 1990;127:724-9.
- Richards JG, Schoch P, Haring P, Takacs B, Mohler H. Resolving GABA_A/benzodiazepine receptors: cellular and subcellular localization in the CNS with monoclonal antibodies. *J Neurosci* 1987;7:1866-86.
- Rivest S, Laflamme N, Nappi RE. Immune challenge and immobilization stress induce transcription of the gene encoding the CRF receptor in selective nuclei of the rat hypothalamus. *J Neurosci* 1995;15:2680-95.
- Rivier J, Rivier C, Vale W. Synthetic competitive antagonists of corticotropin-releasing factor: effect of ACTH secretion in the rat. *Science* 1984;224:889-91.
- Rodriguez de Fonseca F, Rubio P, Menzaghi F, Merlo-Pich E, Rivier J, Koob GF, Navarro M. Corticotropin-releasing factor (CRF) antagonist [D-Phe¹², Nle²¹⁻²⁹, C-MeLeu³³]CRF attenuates the acute actions of the highly potent cannabinoid receptor agonist HU-210 on defensive-withdrawal behavior in rats. *J Pharmacol Exp Ther* 1996;276:56-64.
- Rodriguez de Fonseca F, Carrera MR, Navarro M, Koob GF, Weiss F. Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. *Science* 1997;276:2050-4.
- Roe SY, McGowan EM, Rothwell NJ. Evidence for the involvement of corticotropin-releasing hormone in the pathogenesis of traumatic brain injury. *Eur J Neurosci* 1998;10:553-9.
- Rowe W, Sleverman A, Walker M, Sharma S, Barden N, Seckl JR, Meaney MJ. Antidepressants restore hypothalamic-pituitary-adrenal feedback function in aged, cognitively-impaired rats. *Neurobiol Aging* 1997;18:527-33.
- Roy-Byrne PP, Uhde TW, Post RM, Gallucci W, Chrousos GP, Gold PW. The corticotropin-releasing hormone stimulation test in patients with panic disorder. *Am J Psychiatry* 1986;143:896-9.
- Rueter LE, Jacobs BL. A microdialysis examination of serotonin release in the rat forebrain induced by behavioral/environmental manipulations. *Brain Res* 1996;739:57-69.
- Rupprecht R. The neuropsychopharmacological potential of neuroactive steroids: Cellular and systemic effects. *J Psychiatric Res* 1997;31:297-314.
- Sakaue M, Saito N, Taniguchi H, Baba S, Tanaka C. Immunohistochemical localization of γ -aminobutyric acid in the rat pituitary gland and related hypothalamic regions. *Brain Res* 1988;446:343-53.
- Schinkel AH, Wagenaar E, Van Deemter L, Mol CAAM, Borst P. Absence of the mdrla P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *J Clin Invest* 1995;96:1698-705.
- Schüle R, Rangarajan P, Kliewer S, Ransone LJ, Bolado J, Yang N, Verma IM, Evans RM. Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor. *Cell* 1990;62:1217-26.
- Schulz DW, Mansbach RS, Sproule J, Braselton JP, Collins J, Conman M, Dunaiskis A, Faraci S, Schmidt AW, Seeger T, Seymour P, Tingley FD 3rd, Winston EN, Chen YL, Heym J. CP-154,526: A potent and selective nonpeptide antagonist of corticotropin releasing factor receptors. *Proc Natl Acad Sci USA* 1996;93:10477-82.
- Seasholtz AF, Thompson RC, Douglass JO. Identification of a cyclic adenosine monophosphate-responsive element in the rat corticotropin-releasing hormone gene. *Mol Endocrinol* 1988;2:1311-9.
- Shoham Y, Erb S, Leung S, Buczek Y, Stewart J. CP-154,526, a selective, non-peptide antagonist of the corticotropin-releasing factor, receptor attenuates stress-induced relapse to drug seeking in cocaine- and heroin-trained rats. *Psychopharmacology* 1998;137:184-90.

- Sheng M, McFadden G, Greenberg ME. Membrane depolarization and calcium induce *c-fos* transcription via phosphorylation of transcription factor CREB. *Neuron* 1990;4:571–82.
- Sherman JE, Kalin NH. ICV-CRH alters stress-induced freezing behavior without affecting pain sensitivity. *Pharmacol Biochem Behav* 1988;30:801–7.
- Singh VB, Hao-Phan T, Corley KC, Boadle-Biber MC. Increase in cortical and midbrain tryptophan hydroxylase activity by intracerebroventricular administration of corticotropin-releasing factor. Block by adrenalectomy, by RU 38486 and by bilateral lesions to the central nucleus of the amygdala. *Neurochem Int* 1991;20:81–92.
- Sirinathsinghji DJS. Regulation of lordosis behaviour in the female rat by corticotropin-releasing factor, β -endorphin/corticotropin and luteinizing hormone-releasing hormone neuronal systems in the medial preoptic area. *Brain Res* 1986;375:49–56.
- Skutella T, Probst JC, Criswell H, Moy C, Breese G, Jirikowski GF, Holsboer F. Antisense oligodeoxynucleotide complementary to corticotropin-releasing hormone mRNA reduces anxiety in shuttle-box performance. *Neuroreport* 1994a;5:2181–5.
- Skutella T, Montkowski A, Stöhr T, Probst JC, Landgraf R, Holsboer F, Jirikowski GF. Corticotropin-releasing hormone (CRH) antisense oligodeoxynucleotide treatment attenuates social defeat-induced anxiety in rats. *Cell Mol Neurobiol* 1994b;14:579–88.
- Skutella T, Probst JC, Renner R, Holsboer F, Behl C. Corticotropin-releasing hormone receptor (type I) antisense targeting reduces anxiety. *Neuroscience* 1998;85:795–805.
- Smith GW, Aubry J-M, Dellu F, Contarino A, Bilezikjian LM, Gold LH, Hauser C, Bentley CA, Sawchenko PE, Koob GF, Vale W, Lee K-F. Corticotropin-releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. *Neuron* 1998;20:1093–102.
- Sountag A, Rothe B, Guldner J, Yassouridis A, Holsboer F, Steiger A. Trimapramine and imipramine exert different effects on the sleep EEG and on nocturnal hormone secretion during treatment of major depression. *Depression* 1996;4:1–13.
- Spengler D, Rupprecht R, Phi Van L, Holsboer F. Identification and characterization of a 3',5'-cyclic adenosine monophosphate-responsive element in the human corticotropin-releasing hormone gene promoter. *Mol Endocrinol* 1992;6:1931–41.
- Spina M, Merlo-Pich E, Chan RKW, Basso AM, Rivier J, Vale WW, Koob GF. Appetite-suppressing effects of urocortin, a CRF-related neuropeptide. *Science* 1996;273:1561–4.
- Steiger A, Holsboer F. Neuropeptides and human sleep. *Sleep* 1997;20:1038–52.
- Stenzel-Poore P, Kesterson R, Yeung W, Cone RD, Rittenberg MB, Stenzel-Poore MP. Identification of a novel murine receptor for corticotropin-releasing hormone expressed in the heart. *Mol Endocrinol* 1995;9:637–45.
- Stenzel-Poore MP, Cameron VA, Vaughan J, Sawchenko PE, Vale W. Development of Cushing's syndrome in corticotropin-releasing factor transgenic mice. *Endocrinology* 1992;130:3378–86.
- Stenzel-Poore MP, Heinrichs SC, Rivest S, Koob GF, Vale WW. Overproduction of corticotropin-releasing factor in transgenic mice: a genetic model of anxiogenic behavior. *J Neurosci* 1994;14:2579–84.
- Strijbos PJLM, Rilton JK, Rothwell NJ. Corticotropin-releasing factor antagonist inhibits neuronal damage induced by focal cerebral ischaemia or activation of NMDA receptors in the rat brain. *Brain Res* 1994;656:405–8.
- Sulser F, Vetulani J, Mohley PL. Mode of action of antidepressant drugs. *Biochem Pharmacol* 1978;27:257–71.
- Sutton RE, Koob GF, Le Moul M, Rivier J, Vale W. Corticotropin releasing factor produces behavioural activation in rats. *Nature* 1982;297:331–3.
- Swanson LW, Simmons DM. Differential steroid hormone and neural influences on peptide mRNA levels in CRH cells of the paraventricular nucleus: a hybridization histochemical study in the rat. *J Comp Neurol* 1989;285:413–35.
- Swanson LW, Sawchenko PE, Rivier J, Vale WW. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. *Neuroendocrinology* 1983;36:165–86.
- Swerdlow NR, Britton KT, Koob GF. Potentiation of acoustic startle by corticotropin-releasing factor (CRF) and by fear are both reversed by α -helical CRF (9–41). *Neuropsychopharmacology* 1989;36:165–86.
- Swiergiel AH, Takahashi LK, Kalin NH. Attenuation of stress-induced behavior by antagonism of corticotropin-releasing factor receptors in the central amygdala in the rat. *Brain Res* 1993;623:229–34.
- Takuma K, Matsuda T, Yoshikawa T, Kitanaka J, Gotoh M, Hayata K, Baba A. Corticotropin-releasing factor stimulates Ca^{2+} influx in cultured rat astrocytes. *Biochem Biophys Res Commun* 1994;1103:7.
- Timpl P, Spanagel R, Sillaber I, Kresse A, Reul JMM, Stalla GK, Blanquet V, Steckler T, Holsboer F, Wurst W. Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. *Nature Genet* 1998;19:162–6.
- Tsai GE, Ragan P, Chang R, Chen S, Linnoila ML, Coyle JT. Increased glutamatergic neurotransmission and oxidative stress after alcohol withdrawal. *Am J Psychiatry* 1998;155:726–32.
- Valdenaire O, Giller T, Breu V, Gottowik J, Kilpatrick G. A new functional isoform of the human CRF₂ receptor for corticotropin-releasing factor. *Biochem Biophys Acta* 1997;1352:129–32.
- Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β -endorphin. *Science* 1981;213:1394–7.
- Valentino RJ, Wehby RG. Corticotropin-releasing factor: evidence for a neurotransmitter role in the locus coeruleus during hemodynamic stress. *Neuroendocrinology* 1988;48:674–7.
- Valentino RJ, Foote SL, Aston-Jones G. Corticotropin-releasing hormone activates noradrenergic neurons of the locus coeruleus. *Brain Res* 1983;270:363–7.
- Valentino RJ, Foote SL, Puge ME. The locus coeruleus as a site for integrating corticotropin-releasing factor and noradrenergic modulation of stress responses. *Ann NY Acad Sci* 1993;697:173–88.
- Van Bockstaele EJ, Colago EEO, Valentino RJ. Corticotropin-releasing factor-containing axon terminals synapse onto catecholamine dendrites and may presynaptically modulate other afferents in the rostral pole of the nucleus locus coeruleus in the rat brain. *J Comp Neurol* 1996;364:523–34.
- Vaughan J, Donaldson C, Bittencourt J, Perrin MH, Lewis K, Sutton S, Chan R, Turnbull AV, Lovejoy D, Rivier C, Rivier J, Sawchenko PE, Vale WW. Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. *Nature* 1995;378:287–92.
- Vita N, Laurent P, Lefort S, Chalon P, Lelias MJ, Kaghad M, Le Fur G, Caput D, Ferrara P. Primary structure and functional expression of mouse pituitary and human brain corticotrophin releasing factor receptors. *FEBS Lett* 1993;335:1–5.
- von Bardeleben U, Holsboer F. Cortisol response to a combined dexamethasone-hCRH challenge in patients with depression. *J Neuroendocrinol* 1989;1:485–8.
- von Bardeleben U, Holsboer F. Effect of age upon the cortisol response to human CRH in depressed patients pretreated with dexamethasone. *Biol Psychiatry* 1991;29:1042–50.
- von Bardeleben U, Holsboer F, Stalla GK, Müller OA. Combined administration of human corticotropin-releasing factor and lysine vasopressin induces cortisol escape from dexamethasone suppression in healthy subjects. *Life Sci* 1985;37:1613–18.
- von Bardeleben U, Stalla GK, Müller OA, Holsboer F. Blunting of ACTH response to human CRH in depressed patients is avoided by metyrapone pre-treatment. *Biol Psychiatry* 1988;24:782–6.
- von Bardeleben U, Heuser I, Holsboer F. Human CRH stimulation response during acute withdrawal and after medium-term absten-

- tion from alcohol abuse. *Psychoneuroendocrinology* 1989;14:441–9.
- Wang L, Martinez V, Vale WW, Tache Y. Peripheral injection of corticotropin-releasing factor (CRF) and a CRF-related peptide, urocortin, activate specific brain areas in rats. *Gastroenterology* 1996;110:A1131.
- Webster EL, Lewis DB, Torpy DJ, Zachman EK, Rice KC, Chrousos GP. In vivo and in vitro characterization of antalarmin, a non-peptide corticotropin-releasing hormone (CRH) receptor antagonist: suppression of pituitary ACTH release and peripheral inflammation. *Endocrinology* 1996;137:5747–50.
- Wiedemann K, Holshoer F. The effect of repeated human corticotropin-releasing hormone administration on dexamethasone-suppressed pituitary-adrenocortical activity in healthy subjects. *Biol Psychiatry* 1997;42:882–8.
- Wiersma A, Baauw AD, Bohus B, Kooblaas JM. Behavioural activation produced by CRH but not α -helical CRH (CRH-receptor antagonist) when microinfused into the central nucleus of the amygdala under stress-free conditions. *Psychoneuroendocrinology* 1995;20:423–32.
- Wilner P. The validity of animal models of depression. *Psychopharmacology* 1984;83:1.
- Wilson MA, Biscardi R, Smith MD, Wilson SP. Effects of benzodiazepine agonist exposure on corticotropin-releasing factor content and hormonal stress responses: divergent responses in male and ovariectomized female rats. *J Pharmacol Exp Ther* 1996;278:1073–82.
- Young EA, Akil H, Haskeu RF, Watson SJ. Evidence against changes in corticotroph CRF receptors in depressed patients. *Biol Psychiatry* 1995;37:355–63.
- Zobel A, Yassouridis A, Frieboes R-M, Holshoer F. Cortisol response to the combined dexamethasone-CRH test predicts medium-term outcome in patients with remitted depression. *Am J Psychiatry* 1999; in press.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.